CHAPTER 11

Summary and Conclusion

A brief summary of the important findings obtained from the study is given below:

11.1 Studies on the effects of CnI in normoglycemic and hyperglycemic rats

The results of the dose response study using CnI in diabetic rats indicated that CnI feeding had a significant blood sugar lowering effect in alloxan induced diabetic rats. Feeding CnI at different levels (10, 20 and 40% w/w) to diabetic rats caused a significant decrease in serum glucose and a significant increase in hepatic glycogen. The glucose lowering effect was more in rats administered with 20% CnI indicating its better antihyperglycemic effect. Diabetic rats fed different levels of CnI also showed a significant reduction in the serum HbA₁c and a corresponding increase in the serum levels of total hemoglobin. These effects were also predominant in those animals fed 20% CnI indicating better glycemic control. The results proved that dietary supplementation of 20% CnI was superior in antihyperglycemic action than the other two dose levels.

The effects of pretreatment and post-treatment of 20% CnI on carbohydrate metabolism in alloxan diabetic rats were studied and observed that diabetic rats pretreated with CnI had a moderate effect on serum glucose levels. Whereas, diabetic rats post-treated with 20% CnI showed a significant reduction in the serum glucose level. Treatment with CnI elevated the hepatic glycogen content of diabetic rats. Normal rats fed with CnI also moderately increased the levels of hepatic glycogen storage compared to the normal control. Normal and diabetic rats fed with 20% CnI showed a gain in body weight by 7.07 and 2.1% respectively. CnI pretreatment and post-treatment significantly increased the activities of glycolytic enzymes such as hexokinase, pyruvate kinase and phosphoglucomutase in diabetic rats. Normal rats post-treated with CnI also showed an increase in the activities of these enzymes which were comparable with normal control rats. There was a decrease in the activities of gluconeogenic enzymes like glucose-6-phosphatase and fructose-1, 6 bisphosphatase in
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Diabetic rats post-treated with Cnl. Post-treatment using Cnl to diabetic rats significantly decreased the activity of glycogen phosphorylase which was found to be increased in diabetic control rats. The activity of SGOT, SGPT and ALP were found to be increased in the serum of diabetic control rats, while diabetic rats post-treated with Cnl caused a significant reduction in these enzymes indicating the non-toxic nature of Cnl in rodents. The estimates of AUCglucose values of OGTT indicated that Cnl treatment to diabetic rats showed 57.25% decrease in blood glucose levels. Histopathology of liver and pancreas of diabetic rats treated with Cnl showed only mild degenerative changes compared with the diabetic control group.

11.2 Studies on phytochemical screening and in vitro antioxidant and antiglycation activities of Cnl

The qualitative analysis of Cnl extracts revealed that the methanol extract of Cnl is rich in flavonoids, tannins, phenolic acids and carbohydrates. While, ethanol extract contains phytochemical constituents mainly alkaloids, phenolic acids and resins. Ethyl acetate fraction contains acidic compounds and resin and lacks some of the components which are present in methanol and ethanol extracts. Constituents like saponins, fat and oils were absent in all the three extracts. Quantitative estimation of dried powdered Cnl demonstrated that Cnl is rich in dietary fibers. Protein and carbohydrates were moderately present in Cnl, while fat and ash in least concentrations. Quantitative estimation of minor components in dried powdered Cnl revealed the presence of polyphenols, free amino acids, vitamin C, vitamin E, minerals like sodium and potassium. Results of DPPH radical scavenging assay showed that methanol fraction exerted high antioxidant potential observed from the lower IC\textsubscript{50} values. The methanol and ethanol extracts of Cnl had a scavenging activity on the superoxide radicals in a concentration dependent manner. The methanol extract of Cnl had stronger superoxide radical scavenging activity than ethanol extract due to the presence of more active phytochemical constituents in it. Observations from H\textsubscript{2}O\textsubscript{2} scavenging and hydroxyl radical scavenging assays showed that among the extracts, Cnl methanolic extract was the most potent in scavenging hydrogen peroxide radicals and hydroxyl radicals as evidenced by lower IC\textsubscript{50} values. The difference in antioxidant efficiency observed between the different extracts may be attributed to their phytochemical composition. Results also demonstrated that Cnl methanol and ethanol extracts decreased in vitro fluorescent
AGEs formation. The results suggested that methanolic extract of Cnl possess better in vitro antioxidant and antiglycation potentials compared to the ethanol and ethyl acetate extracts.

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

The results indicated that treatment with Cnl extracts to STZ diabetic rats resulted in a reduction in the blood glucose and fructosamine levels. The concentrations of glucose and fructosamine were much reduced in animals treated with Cnl methanol extract indicating a better efficiency of methanolic extract in glycemic control. Serum insulin and hepatic glycogen levels of rats treated with Cnl methanol extract were higher than those rats treated with ethanol and ethyl acetate fractions. Estimation of AUCglucose values from OGTT revealed 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. Treatment with Cnl methanol, ethanol and ethyl acetate extracts significantly reversed the increasing levels of serum HbA1c in diabetic rats indicating the antiglycative effect of Cnl extracts. The activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in hepatic, cardiac and renal tissues were increased significantly in diabetic rats after treatment with Cnl extracts. Among the three extracts, Cnl methanol extract showed a better increase in these enzyme activities. In addition, Cnl methanol extract treatment significantly increased the concentration of reduced glutathione in liver, heart and kidney of diabetic rats. Cnl methanolic extract administration significantly increased α-tocopherol and ascorbate level in serum of diabetic rats compared to ethyl acetate extract. There was no significant difference observed in the levels of α-tocopherol and ascorbate in the serum of diabetic rats fed with Cnl methanol and ethanol extracts. Diabetic rats administered with Cnl extracts showed a significant decrease in the levels of plasma ceruloplasmin. In the histopathology of pancreas, the damaged β-cells seen after the initial induction of diabetes were no longer observed after treatment with Cnl extracts. The recovery of β-cell damage was especially more pronounced after treatment with methanol extract of Cnl than in the groups treated with ethanol and ethyl acetate extracts.
11.4 Effect of feeding different levels of 80% methanolic extract of CnI (MEC) in diabetic rats

The results from this study demonstrated that diabetic rats fed different levels of MEC showed a significant reduction in the serum glucose concentration. Treatment with MEC significantly increased the levels of liver glycogen in diabetic rats. These effects were predominant in rats treated with MEC at a dose of 200mg. Oral administration of different levels of MEC to diabetic rats caused a significant increase in the levels of serum insulin. Concentration of serum insulin of rats treated with MEC at a dose of 200mg were higher than those rats treated with 100 and 400 mg. Treatment with MEC significantly reversed increasing levels of serum HbA1c in diabetic rats indicating the potential of MEC in long term glycemic control. 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. From the results, it was observed that 200 mg/kg body weight is the most effective dose of MEC, which has potent antihyperglycemic effect in STZ diabetic rats and this dose was selected for further studies.

The effects of MEC on TCA cycle enzymes, advanced oxidation of protein, peroxidation of lipid, erythrocyte membrane stability and inflammatory marker enzymes in diabetic rats were studied and observed that MEC treatment significantly increased the activities of SDH, ICDH, MDH and α-KGDH in the liver of diabetic rats. The results suggested that there was increased mitochondrial oxidative potential and energy production when diabetic rats were treated with MEC. Oral administration with MEC at a dose of 200 mg significantly reduced the levels of serum AOPP in diabetic rats. Diabetic rats treated with MEC showed significantly decreased concentration of TBARS in hepatic, renal and pancreatic tissues and erythrocyte membrane. The activities of superoxide dismutase, catalase and glutathione peroxidase in erythrocyte membrane were increased significantly in diabetic rats treated with MEC. Oral administration of MEC significantly increased the concentration of GSH in the RBC membrane of diabetic rats. This might reflected the antioxidant potency of MEC in cell membranes as in erythrocytes. Diabetic rats administered with MEC showed a significant decrease in the activities of the inflammatory marker enzymes such as 5-
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11.5 Comparative evaluation of MEC and metformin in experimentally induced type 2 diabetes

The results showed that diabetic rats treated with both MEC and the standard antidiabetic drug, metformin showed a significant reduction in the serum glucose concentration. There was no significant difference observed between MEC and metformin on serum glucose lowering potential. Oral administration of MEC and metformin to diabetic rats caused a significant increase in the levels of serum insulin. It was observed that the serum insulin augmenting effect of MEC was comparable with metformin. MEC and metformin treatment to diabetic rats significantly decreased the insulin resistance as evidenced from decreased HOMA-IR values. Treatment with either MEC or metformin significantly increased the levels of liver and muscle glycogen in diabetic rats. The effects of MEC on glycogen levels were comparable to that of the antidiabetic drug, metformin. Both MEC and metformin treatment to diabetic rats reversed the increasing levels of total cholesterol, LDL-C and decreasing levels of HDL-C. There was no significant difference observed between MEC and metformin on serum total cholesterol and low density lipoprotein lowering action. Treatment with MEC and metformin significantly decreased the levels of serum triglycerides in diabetic rats. Administration of MEC and metformin to diabetic rats significantly restored the levels of serum total protein and albumin. The gene expressions of GLUT 4 in muscle and adipose tissues of diabetic rats were found to be up regulated on administration with both MEC and metformin. Feeding diabetic rats with MEC and metformin caused an up regulation of the expression of PPAR-γ gene in hepatocytes and adipocytes. Administration of MEC to diabetic rats significantly down regulated the gene expression of resistin in adipocytes, which were comparable with the effect of metformin. Treatment of diabetic rats with MEC significantly down regulated the mRNA expressions of TNF-α in liver and adipose tissues, which were also comparable with the effect of metformin. Gomori aldehyde fuchsin (GAF) staining of the pancreatic tissue of both MEC and metformin treated diabetic rats exhibited obvious amelioration in histological changes induced by STZ.
11.6 Isolation and characterization of active components present in aqueous fraction of MEC

Analysis of FT-IR spectrum of AF exhibited peaks at 1603 and 1521 cm\(^{-1}\) which appeared due to the presence of aromatic ring system. The C-O bond was represented by peak at 1190 and 1080 cm\(^{-1}\). Mass spectrum of AF exhibited an [M+1]\(^+\) peak at m/z 355, which was indicative of the molecular weight of 354 corresponding to the compound with the molecular formula C\(_{16}\)H\(_{18}\)O\(_9\). The \(^{13}\)C NMR data revealed the presence of sixteen carbon atoms. In the \(^1\)H NMR spectrum, the downfield shift of the resonance of the equatorial proton signal of H-3 indicated that the hydroxyl group on C-3 was acylated with a caffeic acid. HPTLC analysis revealed the presence of chlorogenic acid, which was confirmed by comparing with the known standard. The results of elemental analysis also supported the structure of chlorogenic acid with the molecular formula C\(_{16}\)H\(_{18}\)O\(_9\). Thus, spectral analyses and HPTLC data indicated that chlorogenic acid, an ester of caffeic acid and quinic acid, is present in AF which is one of the bioactive constituents responsible for the antidiabetic effect exerted by Cocos nucifera inflorescence.

11.7 Studies on the effects of AF and CGA on mitochondrial oxidative phosphorylation and lysosomal enzymes in STZ induced diabetes

The results revealed that diabetic rats treated with AF and CGA showed a significant reduction in the serum glucose concentration. The activity of mitochondrial respiratory complex, NADH - dehydrogenase in hepatic and pancreatic mitochondria was significantly increased in diabetic rats after treatment with AF and CGA. The effect of AF and CGA in modulating NADH-dehydrogenase activity was comparable with each other. Diabetic rats treated with AF and CGA showed significantly increased activity of succinate - cytochrome c - reductase in hepatic and pancreatic mitochondria. The activity of electron transport complex, ubiquinol - cytochrome c - reductase in hepatic and pancreatic mitochondria was also significantly increased in diabetic rats treated with AF and CGA. Diabetic rats treated with AF and CGA showed significantly increased activity of mitochondrial cytochrome c - oxidase in hepatic and pancreatic tissues. The activity of ATP synthase in hepatic and pancreatic mitochondria was significantly increased in diabetic rats treated with AF and CGA. There was no
significant difference between AF and CGA on mitochondrial ATP synthase activity. Concentration of ATP in the mitochondria of liver and pancreas was increased significantly after treatment with AF and CGA. Oral administration of AF and CGA significantly decreased the amount of ROS produced in the hepatic and pancreatic mitochondria of diabetic rats. The activities of lysosomal enzymes such as β-glucuronidase, β-glucosidase and β-hexosaminidase in liver tissue were decreased significantly in diabetic rats treated with AF and CGA. Administration of AF and CGA to diabetic rats significantly increased the activities of lysosomal acid phosphatase and cathepsin D in hepatic tissue.

11.8 Mechanisms of Antidiabetic Effects of CGA Isolated from Aqueous Fraction of MEC

Results of MTT cell viability assay showed that AF and CGA exhibited maximum viability to β-cells at concentrations 0.2 mg/mL and 40 µg/mL respectively. AF and CGA enhanced insulin release from pancreatic islets isolated from both normal and diabetic rats. It was observed that AF and CGA inhibited the glucose absorption through intestine as evidenced by the reduced glucose absorption from the medium of in situ intestinal perfusion. Treatment of diabetic rats with either AF or CGA down regulated the mRNA expression of SGLT-1 in small intestine, which was comparable with the effect of metformin on SGLT-1 expression in this tissue. It was observed that, AF and CGA significantly enhanced the glucose uptake by isolated hemi diaphragm. AF and CGA showed 1.6 and 1.83 fold increase in glucose uptake respectively compared to the control in the absence of insulin. The effect of both AF and CGA was potentiated in the presence of insulin and a 2.04 fold increase for AF and 2.2 fold increase for CGA was found in the uptake of glucose. Moreover, CGA seemed to be more effective in enhancing peripheral glucose uptake in the presence and absence of insulin compared to AF. Treatment of diabetic rats with both AF and CGA significantly down regulated the mRNA expression of PTP 1B in the myocytes, which were comparable with the effect of metformin. Treatment of diabetic rats with either AF or CGA up regulated the mRNA expression of IRS 1 in muscle tissue, which was also comparable with the effect of metformin on IRS 1 expression in this tissue. The gene expression of GSK-3 in muscular tissue of diabetic rats was found to be down regulated after treatment with both AF and CGA. Treatment with AF, CGA and metformin to
diabetic rats caused a significant increase in the phosphorylation of AMPK. A significant increase was also observed in the phosphorylation of Akt in the skeletal muscle homogenates of diabetic rats after treatment with AF, CGA and metformin.

In conclusion, the results of the present study indicate that the young inflorescence of coconut possesses significant antidiabetic, antioxidant and antiglycation properties in rats induced diabetes mellitus. It exerts these beneficial effects mainly due to the presence of chlorogenic acid.