CHAPTER 6

SUMMARY AND CONCLUSION

Diabetes mellitus (DM) or simply diabetes is a metabolic disorder clinically characterized by hyperglycemia due to defective insulin secretion, defective insulin action or both. The global prevalence of diabetes in 2014 is 8.3% (387 million people) out of which 46.3% is undiagnosed. The number of diabetic patient is estimated to reach 592 million by the year 2035.

Oral antidiabetic drugs are regrettably associated with unavoidable side effects, sometimes even life threatening. Hence it necessitates the identification of novel drugs which might act in mechanistically distinct way compared to existing drug targets. Scientific investigation on traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and therapeutic approaches. Hence the ethnomedicinal hypoglycaemic plants are being hunted vigorously by the scientific community.

Cassia alata Linn. is commonly known as candle bush and is native of tropical America. The plant is a well recognised ethnomedicinal plant for antidiabetic activity and is practiced by different ethnic communities of North East India. The present study investigates the antihyperglycemic activity of the plant to generate a stronger biochemical rationale for confirming *Cassia alata* Linn. as a good source of antidiabetic drugs.

Preliminary phytochemical Screening results revealed the presence of some of the bioactive compounds like alkaloids, carbohydrates, resins (absent in CAME), flavonoids, terpenoids (absent in CAAE), diterpenes, phenols, tannins (absent in CAAE), proteins and amino acids, Cardiac glycosides (absent in CAEE and CAAE) and steroids. Due to the presence of the above said compounds in CA, a significant antihyperglycemic property of the plant is predicted, which might be due to the single/combined activities of the compound/compounds present.

The phenolic contents of the extracts ranged from 26.46 to 35.08 (mg GAE/g dry extract). TPC of CAEE, CAME and CAAE are found to be 31.56, 35.08 and 26.46 (mg GAE/g dry extract). CAME contained highest TPC followed by CAEE and CAAE. The presence of the phenolic compounds in the extracts might be the reason behind the antidiabetic activity of the plant.

CAME showed the highest DPPH radical scavenging activity, followed by CAEE, then CAAE. However none of the extracts were upto the level of the control ascorbic

acid (AA). For CAEE, CAME and CAAE, IC_{50} values are, 4.2µg/ml ($p<0.005$ significant against AA), 2.4µg/ml ($p<0.005$ significant against AA), 7.06µg/ml ($p<0.001$ significant against AA) respectively and the IC_{50} of positive control ascorbic acid is 0.85µg/ml.

In the concentration range investigation of RPA, the reducing power of the extracts increased linearly with concentration. At 20, 40, 60, 80 and 100µg/ml, the reducing power of CAEE is 0.17, 0.39, 0.59, 0.82, 0.98 respectively; CAME is 0.25, 0.41, 0.65, 0.88, 1.01 respectively; CAAE is 0.13, 0.3, 0.48, 0.76, 0.87 respectively; and AA is 0.87; 0.26, 0.46, 0.71, 0.94, 1.2 respectively. The reducing power was found to be in order of CAME > CAEE > CAAE.

The FRAP (Ferric Reducing Antioxidant Power) values of the three extracts were: 22.38mmol FeII/mg of extract for CAEE, 25.87mmol FeII/mg of extract for CAME and 18.32mmol Fe II/mg of extract for CAAE. The FRAP value of CAME is greater than CAEE by 13.49% and CAAE by 29.18%.

CAME showed the higher nitric oxide radical scavenging activity then CAEE and CAAE but not so effective as AA (positive control). The IC_{50} value of CAME and CAEE are 5.47µg/ml and 5.76µg/ml respectively; which is significantly ($p<0.05$) different from IC_{50} of AA. IC_{50} of CAAE (8.94µg/ml) is significantly different from AA (2.19µg/ml) at $p<0.01$.

There was a strong correlation between the TPC and antioxidant activity of the extracts. The strongest correlation was observed between the DPPH radical scavenging activity and TPC of the extracts (R^2=0.999). FRAP value and Reducing Power activities of the extracts are also highly correlated with TPC with R^2=0.996 and R^2=0.955 respectively. However NRSA and TPC of the extracts showed the lowest correlation (R^2=0.887) as compared to other assays.

In α-amylase and α-glucosidase inhibitory test, at the concentration range of 100, 200, 300, 400 and 500µg/ml the extracts exhibited α-amylase inhibitory activity in a dose dependent manner. In case of α-amylase inhibitory test, IC_{50} value of CAEE, CAME, CAAE and acarbose (positive control) are 262.62µg/ml, 235.58 µg/ml, 409.74 µg/ml and 189.33 µg/ml respectively. In case of α-glucosidase inhibitory test, IC_{50} value of CAEE, CAME, CAAE and acarbose are 313.5µg/ml, 202.5 µg/ml, 464.29 µg/ml and 129.09 µg/ml respectively. Polyphenolic compounds present in the extracts might be responsible for the inhibitory potential base on the fact that polyphenolic compounds

in plants inhibit the activities of carbohydrate digestive enzymes because of their ability to bind to proteins. The results showed that CA is a source of inhibitory drugs of carbohydrate digesting enzymes which further gave a clue that *in vivo* antidiabetic activity of the extracts may be due to the inhibition of the above enzymes.

In acute toxicity test (14 days) of the extracts, there was no sign of tremors, convulsions, salivation, diarrhoea, lethargy, sudden or drastic decrease of body weight and coma. And also there were no changes in eyes, respiratory circulation, sleep, etc.

In Oral Glucose Tolerance Test, the effect of CAEE, CAME and CAAE on OGTT in percentage change in BGL at 30min, 60min, 90min and 120min, from initial/baseline BGL (0min) are – 91.42%, 60.87%, 46.72% and 40.85% respectively in NC mice; 84.85%, 53.94%, 42.11% and 21.90% respectively in GTNM; 85.33%, 55.51%, 47.44% and 39.17% respectively in EETNM; 87.56%69.02%, 49.53% and 27.20% respectively in METNM; 88.66%, 71.89%, 50.34% and 43.28% respectively in AqETNM. The effect of CAEE, CAME and CAAE on OGTT in STZ induced diabetic mice, in percentage change in BGL at 30min, 30min, 90min and 120min, from initial/baseline BGL (0min) are – 66.51%, 57.43%, 52.07% and 46.38% respectively in DC mice; 53.51%, 12.75%, 1.50% and -12.50% respectively in GTDM; 53.01%, 32.56%, 16.36% and 5.92% respectively in EETDM; 52.08%, 27.10%, 6.87% and -2.62% respectively in METDM; 55.21%, 30.65%, 10.77% and -0.33 respectively in AqETDM. Among the extracts, CAME showed the maximum tolerance for glucose. The extracts showed a better inhibitory activity, compared with the diabetic control suggesting that the extracts could decrease the postprandial glucose level probably by inhibiting the activity of α-amylase and α-glucosidase, or might be by enhancing the secretion of insulin in response to glucose load and increased peripheral utilization of glucose. Methanol, ethanol and aqueous extracts of the said plant enhanced glucose utilization. The said extracts were able to reduce blood glucose level on STZ induced diabetic mice. It reveals the positive effect of extracts in maintaining glucose homeostasis in mice.

Oral administration of CAEE, CAME and CAAE to diabetic mice for 21 days continuously caused a significant decreased in blood glucose level, with maximum fall observed on 28^{th} day of treatment suggesting a time dependent hypoglycaemic effect of the extracts. The groups treated with 400mg/kg body wt. dose of extracts was found to be more effective than the groups treated with 200mg/kg body wt. dose of

extracts revealing a dose dependent hypoglycaemic effect. In both the doses, the percentage of decrease of BGL was highest in CAME followed by CAEE and then CAAE, revealing a better hypoglycaemic activity in CAME. The glucose lowering potential of the extracts was compared with that of the standard glibenclamide. Oral administration of CAME, CAEE and CAAE to diabetic mice can bring the elevated blood glucose level to normoglycaemia, though the effective nature of the extracts was less intensive and sudden than the standard drug glibenclamide. The hypoglycaemic activity of CAME is higher than CAEE and CAAE.

After *in vivo* antidiabetic activity treatment of the extracts the increased serum levels of TG and TC were significantly suppressed, while the decreased serum HDL levels were significantly improved after treatment with CAME, CAEE, CAAE and glibenclamide at $^{a}P<0.001$, $^{b}p<0.001$ and $^{c}p<0.001$ respectively when compared with diabetic control. Given this result, it appears that the extracts have a transitory lowering activity on lipid levels in serum. This observed result suggest favourable effects of CA on hyperlipidemia. The beneficiary effects of the CA extracts in improving lipid metabolism which is comparable with that of the standard glibenclamide.

Decrease in total protein content was observed in diabetic mice which might be due to excessive catabolism of protein to afford amino acids for gluconeogenesis. CAEE and CAAE treatment normalized the total protein in diabetic mice, suggesting its medicinal role on kidney function.

Treatment with both the doses (200, 400 mg/kg) of CAME, CAEE, CAAE and glibenclamide (10 mg.kg dose) in diabetic mice decreases the SGOT and SGPT activities significantly ($p<0.001$), increases the hepatic glycogen as compared to diabetic control mice. Treatment of the diabetic mice with CAEE and CAAE reduced the concentration of these enzymes in serum compared to the diabetic control which indicates the recovery of liver tissue in treated mice.

Haematoxylin and eosin stained pancreatic sections of normal mice showed Langerhans Islets (LI) with normal cellular structure which are distinctively surrounded by the pancreatic acini (PA). Langerhans Islets were almost completely destroyed leaving empty space in diabetic mice. In glibenclamide, CAME, CAEE and CAAE treated mice the Langerhans Islets maintained regular structure.

In liver sections of diabetic mice, infiltrations of inflammatory cells through hepatic tissue are seen indicating injury of the liver tissue. Normal radiating forms of the hepatocytes are seemed to be distorted. Examined sections of treated mice showed the CA's ability to reduce cell necrosis and helped in retaining the regular form of cellular arrangement around the central vein. Kidneys of untreated diabetic mice showed narrow Bowman's space due to infiltration of the inflammatory cells. However the treated mice showed fewer infiltrations of inflammatory cells. The regenerative effect of the pancreatic cells due to extract administration may enlighten the positive effects of this agent on the production of insulin. It appears that CAME, CAEE and CAAE treatment to diabetic mice preserved the cellular function by its beneficial effects.

CAME increased insulin release from MIN6 β-cells in a dose dependent manner over the concentration gradient (0.01-25mg/ml). At 0.01, 0.05, 0.1, 0.5 1, 5, 10 and 25 mg/ml, the measured insulin secretion enhanced by CAME were 0.095, 0.195, 0.16, 0.29, 0.8, 1.2, 1.35 and 1.61 ng/50.000 cells/20min; and measured insulin secretion enhanced by gliclazide were 0.063, 0.18, 0.11, 0.24, 0.86, 1.12, 1.24 and 1.35 ng/50.000 cells/20min respectively. In a medium supplemented with 2.5mM Ca^{2+}, the effective insulin secreting potential of CAME (2.32 fold increase when compared with the control) was lower than the effective insulin secreting activity of gliclazide (5.16 fold increased when compared with the control), indicating a high Ca^{2+} dependent activity of gliclazide.

The chromatogram of CAME by GC-MS showed 16 peaks revealing 16 compounds. On comparison of the mass spectra of the constituents with the NIST library, the sixteen phytocompounds were characterized and identified. Out of the sixteen compounds identified, some compounds are found to be involved in antidiabetic activity of the plant, based on the literature survey. 3-O-Methyl-D-glucose (3-OMG), is effective in reducing the toxicity of streptozotocin (SZ). Considering the literature of 3-OMG and relating its activity with bioactivity test result, it appears that the toxicity of STZ was greatly reduced in CAME treated mice. Thus the reason behind significant antihyperglycaemic activity of CAME can be partially attributed to 3-OMG. Palmitic acid acutely stimulates insulin-induced glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells probably by enhancing the sensitivity of muscle cells to insulin. Hence it appears that palmitic acid present in CAME at lower concentration might have positive influence on diabetes treatment. Phytol is another

important compound identified, which contain many beneficial effect on health. Phytol of natural or synthetic origin is used as active ingredient in formulations to lower serum levels of triglycerides and/or cholesterol and the presence of phytol in CAME explained the probable mechanism behind the TG, TC lowering activity of CAME. The presence of phytol in CAME probably justified the antidiabetic activity of CAME. A diet enriched in stearic acid protects against the progression of type 2 diabetes in leptin receptor deficient mice (DB/DB). This would increase pancreatic islet fatty acid content and increase insulin exocytosis. The presence of strearic acid (0.45% concentration) in CAME justified the effective insulin secretion potential of CAME which further adds to the intensity of antihyperglycemic activity of CA.

The dendrogram derived from RAPD profiles (Fig 4.25) shows that the samples F1 and F3 are most closely related with a similarity level of 0.12. However the sample F2 showed clear divergence and showed no similarity with the other two plant samples and appeared separately in the phylogenetic tree. The RAPD profiling data and the phylogenetic tree is in accordance with the phenotypic data. The sample F1 and F3 belong to same species *Cassia alata* Linn. F1 and F3 were the same plant species collected from different places with varied climatic conditions. F2 which showed some degree of variation from F1 and F3, belongs to a different species *Cassia tora* Linn. but of the same genus. The RAPD fingerprint of F1 and F3 developed, though of same species showed different pattern.

Considering the traditional knowledge with the present scientific investigations, it can be concluded that *Cassia alata* (L.) possessed antioxidant and antihyperglycemic activity and which if extensively studied could provide many chemically remarkable and biochemically active antidiabetic drugs.