CHAPTER-VI

SUMMARY AND CONCLUSION

Diabetes mellitus is a complex and a multifarious group of disorders that are characterized by shortage or lack of insulin secretion or action or reduced sensitivity of the tissue to insulin leading to a metabolic disorder of multiple aetiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. Persistent hyperglycemia or high glucose levels leads to certain life threatening long term complication causing damages and failure of various organs and cells especially eyes, kidneys, nerves, heart and blood vessels.

Currently type2 diabetes is the most common type of diabetes mellitus affecting more than 371 million people globally in the year 2012 comparing to 366 million in 2011 and is expected to rise to 552 million by the year 2030. India is presently home to 62 million diabetics, an increase of nearly 2 million in just one year. By 2030, India's diabetes numbers are expected to cross the 100 million mark.

Management of diabetes mellitus without any side effects is still a challenge to the medical system as used of oral hypoglycemic drugs are restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. This leads to an increasing search for improved antidiabetic drugs. Besides the therapy based on chemotherapeutic agents, the present century has progressed towards naturopathy. Thus medicinal plant plays an important role in treatment and management of diabetes.

In India, indigenous remedies have been used in the treatment of diabetes mellitus since old age time. And a wide and diverge range of plants have been reported in Indian literature.

Eugenia operculata Roxb. is a small or medium sized evergreen tree widely used by the people of Manipur for its various pharmacological properties and also to meet their primary health care needs.

This study has been undertaken to evaluate the activities of crude extract of *E.operculata* leaves on invitro α-amylase, α-glucosidase inhibitory effect and in vivo oral glucose tolerance test, blood glucose level, body weight, hypolipidemic,

glycosylate hemoglobin, liver glycogen content in STZ induced diabetic mice including histopathological and microscopical studies of mouse pancreas. In addition invitro antioxidant activity were also determined.

The α-amylase inhibitory activity of *E.operculata* extract showed that of all the extracts, ethanol and aqueous extract exhibited maximum inhibitory activity with IC50 28.32 µg/ml and 38.82 µg/ml in comparison to 34.83µg/ml of acarbose. Also, *E.operculata* leave extract investigated in the current study demonstrated dose dependent yeast α-glucosidase inhibitory activity. Of all the extract treated aqueous extract showed maximum inhibitory with IC50 38.61 µg/ml followed by ethanol extract, IC50 54.25µg/ml but lesser then the reference drug acarbose with IC50 30.57µg/ml. Thereby considering *E.operculata* as a promising material for preventing and treating diabetes as these enzyme plays an important role in carbohydrate digestion and glucose adsorption leading to high blood glucose level.

Oral glucose tolerance test studies indicated rise of blood glucose level within 30 minutes in all the groups, both normal and diabetes. After 30 minutes reduction of blood glucose level was observed in all the groups. After 30 minutes the groups treated with *E.operculata* extract and glibenclamide significantly suppressed ($p<0.05$) rise in postprandial blood glucose level. The decline in the level of blood glucose reached its maximum after 120 minutes ($p<0.01$).

Oral administration of ethanol and aqueous extract and glibenclamide to diabetic mice for 21 days significantly ($p<0.05$) reversed their fasting blood glucose level to normal. From all the groups *E.operculata* ethanol at the dose of 250mg/kg b.w showed maximum fall of glucose level and found significant ($p<0.001$) compared to glibenclamide treated group. *E.operculata* ethanol and aqueous treated for 21 days also showed that body weight, LDL, total cholesterol, triglyceride, liver glycogen were found to be significantly ($p<0.05$) increased whereas glycated hemoglobin was significantly ($p<0.06$) decreased in the diabetic mice.

Histopathological and electron microscopical studies of STZ induced diabetic mice pancreas reveals destruction of pancreas with severe alteration of beta cells, vacuolization and lysis of the entire cytoplasm and the absence of insulin granules. Also it showed advanced fibrosis which originated in the surrounding capsules with

collagen fascicles that intruded among cells, isolating and in some area destroying them. In *E.operculata* extract and glibenclamide treated groups more than moderate protection against the STZ induced beta cells destruction are observed even though the destructive effect are still present as dilations of GER and of the perinuclear space and some nuclei with irregular outline or abnormal space. Numerous insulin granules both mature and immature and many mitochondria were seen in the cytoplasm of beta cells. There are two possible explanations of these findings. First, *E.operculata* extract may exert its effect by preventing the death of beta cells or second, it may permit recovery of partially destroyed beta cells thereby increasing insulin secretion.

Phytochemical screening of ethanol and aqueous reveals the presence of flavonoids, carbohydrates, resins, terpenoids, phenol, tannins in the both extract. The presence of alkaloids and cardiac glycoside were seen in aqueous extract only. Bioassay guided fractionation leads to the isolation of four active compounds namely Quercetin-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, Kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, Quercetin-3-O-β-D-glucopyranosyl-(1→6)-α-L-rhamnopyranoside and kaemferol-3-7-O-α-diahamnoside from ethanol extract.

Thus it can be concluded on the basis of the current study that the extract of *E.operculata* were found to be safe and it was confirmed by acute and repeated dose toxicity studies. The extract also showed a comparative antidiabetic and antioxidant activity. The antidiabetic activity of *E.operculata* extract might be due to the inhibition of digestive enzyme or normalization of pancreatic cells stimulating insulin secretion or can also be due to the effect on sensitizing insulin and insulin receptors in diabetic mice. Hence *E.operculata* could be considered for the preparation of potent antidiabetic drugs.

Limitations and future prospective of this study:

Although this study provides promising effect of *E.operculata* Roxb. extract against experimentally induced diabetic mice by lowering blood glucose level and normalizing lipid parameters, the precise mechanism of this effect is still to be determined. And also of the four compound characterized from the active fraction, antidiabetic properties of Quercetin-3-O-β-D-glucopyranosyl-(1→6)-α-L-

rhamnopyranoside and Kaemferol-3-7-O-α-diahamnoside has already reported however no such report has been found of the remaining two compound, Quercetin-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, Kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside. So this study warrants further research on the antihyperglycemic properties of *E.operculata* Roxb.