CHAPTER 5

CONCLUSIONS

- High frequency shoots were obtained from the shoot tip explants at 1.5 mg/l BAP in combination with 25mg/l AdS. Elongation of the juvenile shoots was observed in 0.3 mg/l GA$_3$. Rooting was best observed within 7 days in 1.0 mg/l IBA. The rooted shoots were acclimatized at 70% survival rate. The system was rapid starting with the initiation of shoots and ending with the transplanting of in vitro regenerants to soil takes 3-4 months duration.

- The RAPD analysis was performed to study the genetic stability of the in vitro regenerated plants. Five in vitro regenerated plants along with a ex vitro raised was screened with eight primers (OPA20, OPA7, OPA9, OPA10, OPA11, OPA13, OPA18 and OPA19) out of which two primers (OPA7 and OPA10) generated 13 scorable bands from 400 bp to 1600 bp in size and 350 bp to 1100 bp in size. The banding patterns of the in vitro regenerants did not show any polymorphism.

- Preliminary phytochemical investigation revealed the presence of alkaloid, phenols, flavanoids, saponins, glycosides and tannins. The total flavanoid content was found to be higher than the total phenol content.
• In all the *in vitro* antioxidant models tested the percentage of inhibition increased with increase in concentration of EtALL extract which was closely comparable with vitamin C and E. The cell viability assay showed the toxicity of the EtALL extract was higher at 100 and 200µg/ml in 3T3-L1 cell line. Hence, the dosage of EtALL extract was fixed as 25, 50 and 75 µg/ml for both insulin mimicking and sensitization activity. The insulin sensitization activity was found to be better than insulin mimicking activity at 25 µg/ml.

• The acute toxicity studies were conducted in the wistar male albino rats, the dosage for evaluation of the antidiabetic activity was fixed as 200 and 400 mg/kg b.w. Oral administration of EtALL extract at 400 mg/kg b.w. showed significant improvement in biochemical parameters and enhanced glucose rate in OGTT.

• Induction of diabetes by streptozotocin increased the blood sugar level and altered the biochemical parameters. There was increased body weight gain with reduced food and water intake on administration of EtALL extract at 400 mg/kg b.w. The blood glucose level decreased with increase in plasma insulin activity.

• The Hb and HbA1C levels decreased where as muscle and liver glycogon level increased significantly by the administration of EtALL at 400 mg/kg b.w. The activities of carbohydrate metabolizing enzymes such as hexokinase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase was reversed from diabetic related changes on administration of EtALL at 400 mg/kg b.w.

• The levels of HDL-c and total protein increased whereas total cholesterol, triglycerides, LDL-c decreased on administration of
EtALL at 400 mg/kg b.w. The levels of urea, uric acid, and creatinine were also decreased with the increased level of albumin and A/G ratio.

- The diabetic TBARS and hydroperoxides levels decreased accompanied by the increase in the level of antioxidant enzymes such as SOD, CAT, GPx and GST. There was an increase of GSH and reduced glutathione on administration of EtALL extract at 400 mg/kg b.w. The levels of serum liver marker enzymes such as AST, ALT, LDH, ALP and ACP were retrieved closely to normal on administration of EtALL at 400 mg/kg b.w.

- Histological observation of liver, kidney and pancreas of diabetic rats indicated fatty infiltration, shrinkage of islets of langerhans in pancreas. Sinusoidal congestion, fatty degeneration in liver. Fatty infiltration and parenchymal inflammation in kidney. Treatment of EtALL at 400 mg/kg b.w. dosage for 28 days markedly reduced all the pathological changes.

- The EtALL extract was subjected to column chromatography by stepwise gradient method with solvents petroleum ether, chloroform, ethyl acetate and methanol. Screenings of isolated active fractions (fraction I to V) were performed in 3T3-L1 preadipocytes cell line and lipogenesis was measured by Oil-Red-O-stain. The fraction-IV showed significant lipogenesis activity was further subjected to structure elucidation studies in NMR (\(^1\text{H} - ^1\text{C}\) and \(^1\text{H}-^1\text{H}\) COSY), IR and ESI-MS. The active compound was found to be Dimethyl 3, 3', 4, 4'-tetrahydroxy- \(\delta\)-truxinate (DT\(\delta\)T) which belongs to truxinic acid derivative.
DTδT (bioactive compound) showed significant insulin sensitization than mimicking activity at 25 µg/ml. The lipogenic activity was also found to be significant at the same concentration.

The RT-PCR analysis revealed the down regulation of the mRNA expressions in both the genes (PPAR-γ and C/EBP-α) at 25 µg/ml concentration of DTδT.

The docking studies were performed in DTδT and rosiglitazone using PPAR-γ and C/EBP-α receptors. The docking score of DTδT were found to be relatively less when compared with rosiglitazone in both the receptors. However there is a presence of hydrogen bonding in both the receptors only in DTδT which may be further structurally modified to serve as a lead molecule in the discovery of novel antidiabetic drug.

This promising, cost-effective protocol for micropropogation will help in the rapid multiplication, large-scale production and conservation of Andrographis lineata. This method for plant regeneration could be helpful for the study of phytomedicine production, somaclonal variation and genetic transformation. The invitro and invivo evaluation of diabetic model study confirmed the presence of antidiabetic activity of the crude ethanolic leaf extract of Andrographis lineata. Carbohydrate metabolism and differentiation of 3T3-L1 adipocytes are associated with diabetes. PPARγ and C/EBP family are key transcription factors for adipocytes differentiation. In this study, the expression of PPAR-γ and C/EBP-α induced by DEX, IBMX and insulin was inhibited together with adipogenisis measured by Oil-Red-O- staining. So it is appears that DTδT decreased fat accumulation by inhibiting the expression of PPAR-γ and C/EBP-α. We therefore suggest that its effect on glucose uptake might be one of the possible mechanisms for its anti-diabetic action.