CHAPTER 5

CONCLUSION

The present study is focused on the identification of active components from biologically important medicinal plants and validating their potential by mapping their mechanism of action and eventually leading to the development of new drugs, which can be promoted either as alternate or complementary medicines. In this regard the plant *Calotropis procera* was selected for column fractionation and purification based on preliminary bio-screens.

Bioassay-guided fractionation of *Calotropis procera* led to the identification of a bio-active pure compound. The molecular structure of the pure compound was elucidated. The present thesis highlights the ability to identify ethnobotanical leads by chemical isolation techniques to study various aspects of cell signaling cascades to arrive at molecules with potent anti-inflammatory activity. The bioactivity based screens used in this study involves measuring proliferation of PBMC and JURKAT T-cells using $[^3]$H Thymidine and monitoring known key mediators of inflammatory disorders, namely TNFα, IL-1β, NO, iNOS, COX-2, etc at a secondary level, with a view to postulate the possible mechanism by which these extracts reduce immune disorders.

This study of the crude extract and purified compound of *Calotropis procera* formed a surmise leading to following revelation:
The expression pattern and the anti-inflammatory effect of *Calotropis procera* on various mediators, that regulate important biological activities of cells in inflammation and those that are up regulated in inflammatory disorders, were studied.

Lymphocyte proliferation was significantly inhibited by the Crude extracts and pure compound of *Calotropis procera*.

The ethyl acetate crude extract and pure compound of *Calotropis procera*, significantly inhibited pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, IL-8, IFNγ and IL-12.

Observations on pro-inflammatory mediators PLA2, COX-2 and NO showed that *Calotropis procera* pure had moderate effect on PLA2, while it down regulated COX-2, NO production and iNOS expression. Molecular modelling of the pure compound with COX-2 is indicative of possible competitive binding mechanism, and hence the consequent anti-inflammatory property.

*Calotropis procera* down regulated expression of Th1 cytokines, IL-12 and IFNγ. This down regulation may in part be contributed by its capacity to up regulate IL-10. IL-10 is anti-inflammatory and is a negative regulator of immune response under physiological condition.

The present study demonstrates that the anti-inflammatory effect of *Calotropis procera* pure is likely the consequences of inhibition of JNK and p38 MAP kinase pathways, which thus can affect AP-1 expression and subsequently brings about down regulation of inflammatory mediators. Observations on the transcription factor NF-κB showed marked inactivation of the transcription factor under LPS induced condition. Hence regulation of
inflammatory mediators observed on treatment with *Calotropis procera* crude and pure is thought to be through its effect on MAPK signaling cascade and inhibition of NF-κB activation

The increase in STAT-3 and SOCS-3, further confirmed the effect of *Calotropis procera* pure on up-regulation of Th2 cytokines thus the down regulation of macrophage mediators and Th1 type responses.

A study focussing on the effect of molecules of herbal origin on specific targets to counter the harmful effects of immune hyper activation would go a long way in preventing many autoimmune and other immune related disorders. The studies carried out on the plant extracts from *Calotropis procera* suggested the presence of potent compounds that were capable of inducing anti-inflammatory effects via specific signal cascades involved in the complex inflammatory machinery. These studies also suggest that the pure compound may be analysed further to evaluate their potency, to serve as valuable anti-inflammatory drugs or as a template for the design of prospective new anti-inflammatory drug candidates.

5.1 SCOPE FOR FUTURE DIRECTIONS

The present work highlights the anti-inflammatory potential of the plant *Calotropis procera*. The bioactive lead isolated from it, 28-norolean-12-ene-3,17-diol has an effective anti-inflammatory response. The pharma industry today is screening drug candidate from the natural product pool; like never before. The premise that natural products throws out unique or known metabolites with previously unknown bioactivity has been validated. The *in silico* approach adapted is one of the major screening. Further structural modifications to the bioactive pure would provide a better insight into increase or decrease in its anti-inflammatory potential.