CHAPTER 9

CONCLUSIONS AND FUTURE SCOPE

Objectives of the Chapter

- *To discuss the conclusions*
- *Suggestions regarding future work*

The thesis has reviewed the pathophysiology and therapeutic strategies of cerebral ischemia, rodent model of global cerebral ischemia, role of molecular docking for screening of inhibitors, chlorogenic acid and use of impedance spectroscopy for diagnosis of ischemic stroke in chapter 2. The WS phytochemicals was screened using molecular docking and results revealed that chlorogenic acid has potential to inhibit NMDAR, nNOS and gelatinase which is discussed in chapter 3. Chapter 4 demonstrated that CGA reached to the brain in higher concentration at 30 minutes of administration when administered via intranasal route compared to intravenous route. The concentration of nitrate was successfully determined in various brain regions and CSF of rat using a simple HPLC-UV method in chapter 5. Chapter 6 demonstrated that change in brain impedance during ischemia-reperfusion state has a relation with changes of brain electrolyte concentration. Chapter 7 confirmed that hypotension was induced using vasodilator drug when coupled with BCCAO for one hour followed by one hour of reperfusion damage the BBB and developed cerebral infarction in rats. Chapter 8 demonstrated that CGA confer neuroprotection in global cerebral ischemic rat model by inhibition of NMDAR and nNOS and by suppressing the expression of TNF-alpha, iNOS and caspase-3.

9.1 Conclusion

Very first, *in silico* study provides significant evidence that chlorogenic acid can be developed as potent multi-target inhibitor as it displayed extensive hydrogen bounding and hydrophobic

interaction with all molecular targets (NMDAR, MMP-2, MMP-9, nNOS and iNOS) compared to their inhibitors. It also showed higher selectivity for nNOS and iNOS over eNOS. Therefore, its pharmacokinetics and brain distribution profile were analyzed in vivo using rats after IN and IV administration. The brain distribution studies suggest that nasal route mediates direct nose-to-brain transportation of CGA efficiently as compared to IV administration. Increased penetration and high exposure of the CGA in the brain tissue as well as significantly higher drug targeting efficiency (DTE= $AUC_{brain/}AUC_{plasma}$) were found in IN group as compared to the IV group. These studies prove that IN delivery system of CGA can be a promising approach for use in the treatment of ischemic stroke.

An optimized HPLC-UV method for determination of nitrate in rat cortex, cerebellum hippocampi, and CSF was successfully developed. The advantages of this method are simple mobile phase composition, rapid analysis and reproducible results. The method is suitable to analyze nitrate in the brain tissues and CSF in biochemical and pharmaceutical research.

The bio-impedance study demonstrated the changes in brain impedance during ischemia-reperfusion with corresponding changes in electrolyte concentrations in rat brain. The extracellular and intracellular ionic concentrations determine the characteristic behavior such as resistivity, conductivity, and permeability of the brain tissue. The impedance changes in *in-vivo* condition has a relation with the characteristic behavior of impedance change observed in the *in-vitro* study. The present study establishes that the changes in bio-impedance across rat brain can be inter-related with the changes in both intracellular and extracellular ionic concentrations using impedance modelling.

A simple hypotensive/ischemic rat model is developed. The study successfully demonstrates that the administration of amlodipine and metoprolol can induce hypotension in rats. The

hypotension coupled with bilateral common carotid artery occlusion for 1h followed by 1h of reperfusion is sufficient to produce cerebral infarction, BBB disruption, and histopathological damage. This ischemic rat model is simple and has higher reproducibility as well as suitable for biochemical and molecular studies as well as for the evaluation of neuroprotective agents. At a later stage of the thesis, neuroprotective potential of chlorogenic acid was validated using global cerebral ischemic rat model. The findings suggest that CGA can be used as a potent neuroprotective agent in global ischemic condition. After treatment, it confers neuroprotective effects mainly by downregulating the expressions of proinflammatory and apoptotic markers in the cortex as well as by reducing the cerebral lesion, BBB disruption, and brain edema. The neuroprotective ability of CGA might be attributed to its dual inhibition of NMDAR and nNOS that is supported by *in vivo* and molecular docking studies. From the above findings, it can be concluded that the ability of CGA to inhibit and downregulate different molecular markers of cerebral ischemia can be exploited for designing a multi-target inhibitor in neurotherapeutic for combating cerebral ischemic stroke.

9.2 Future directions

In the near future, the nitrite determination, brain impedance-electrolyte correlation, hypotensive/ischemic model development and neuroprotective potential evaluation of chlorogenic in the present work can further be extended. The direction of work may be as follows:

- A significant changes were observed in the nitrite concentration with respect of time and different sample storage conditions (-20^{o}C, -80^{o}C and 4^{o}C). The nitrite concentration decreases significantly within 2 hours making the quantification difficult.

Therefore, this limitation can be overcome by performing a study to stabilize the nitrite level in the biological sample for its determination by HPLC UV.

- Further studies regarding precise quantitation of electrolyte concentrations in extracellular and intracellular space during occlusion and reperfusion of rat brain will be conducted for the better understanding of the brain impedance changes during ischemia and reperfusion state.
- The hypotension coupled with bilateral common carotid artery occlusion for 1h followed by 1h of reperfusion is sufficient to produce cerebral infarction, BBB disruption, histopathological damage as well as brain cell death. However, a study with more than 1h of reperfusion shall be conducted to analyze the behavioral changes in rats.
- The neuroprotective potential of chlorogenic acid will be extended to evaluate its multi-target inhibition efficacy for calpains, PARP-1, RIP-1K, Aquaporin, ASIC, NOGO A and other molecular mediators of neurodysfunction in neurodegenerative diseases.
- A study using an X-ray crystallography technique should be performed to map the interactions between the CGA and active site of MMP-2/9, NMDARs, nNOS, and Calpains.
- . The tissue distribution study of CGA after administration via IN and IV route will help to understand the ADME profiling of CGA.
- Different kinds of toxicity in vivo studies should be performed to evaluate the side effects of CGA after IN administration.
- The Histopathology study of the nasal cavity will also be informative at different IN dosages.