CHAPTER 8

CONCLUSIONS AND FUTURE STUDIES

Chapter Highlights

- ***Discussion of the conclusions***
- ***Future direction of work***

The present study tries to identify therapeutic targets for designing suitable inhibitors which can be further developed into neurotherapeutics for ameliorating the global cerebral ischemia induced pathophysiological damage. Extensive literature review reveals the involvement of PARP-1 and RIPK1 as major molecular mediators of caspase independent cell death pathways of cerebral ischemia pathophysiology, parthanatos and necroptosis, respectively. Inhibition of these two molecular mediators reportedly improves brain damage caused by ischemic. The current work focusses on virtual screening of suitable inhibitors against PARP-1 and RIPK1 by using molecular docking approach and further *in-vivo* evaluation of the screened compounds. Besides, the study also explores the ability of the neuropeptide prolactin in conferring neuroprotection *in-vivo* against global cerebral ischemia.

Phytochemicals from *Withania somnifera*, an *Ayurvedic* herb, were screened virtually to identify potent inhibitors of PARP-1. The binding affinity, hydrogen bonding and hydrophobic interaction pattern of the screened phytochemicals against PARP-1 were compared with the similar parameters of reported inhibitors of PARP-1. The study revealed that five phytochemicals of *W. somnifera* bind to the active site of parp-1 with affinity higher than the reported the inhibitors and exhibited extensive hydrogen bond formation and hydrophobic interactions with the active site amino acid residues. Among these high affinity

phytochemicals, Withanolide A demonstrates low binding energy while exhibiting an almost similar pattern of hydrogen bonding and hydrophobic interactions as that of Talazoparib, an inhibitor of PARP-1 presently in clinical trials. Based on the results of *in-silico* screening, neuroprotective effect of Withanolide A was further studied in global cerebral ischemia model of mice. Results revealed that Withanolide A ameliorates ischemia induced cerebral infarction, restores BBB damage and reduces cerebral edema. The phytochemical also decreased elevated neurotransmitter (glutamate and GABA) levels in different brain regions and also lowers the calcium nitrate concentrations. Withanolide A also rescues tissue damage induced due to ischemic insult and reduces apoptotic and necrotic cell death.

RIPK1 is another enzyme modulating ischemic cell death cascade and has been recently reported to act upstream of PARP-1, thereby affecting activation of PARP-1 and further cell death process. Molecular docking of RIPK1 with estrogen group of hormones identified Estetrol as a potent inhibitor of the enzyme and further *in-vivo* studies showed that Estetrol can combat cerebral ischemia pathophysiology by reducing cerebral infarction, restoring BBB damage and cerebral edema, modulating neurotransmitter levels, cellular calcium and nitrate levels. Histopathological studies suggested that Estetrol also protects the brain cortical tissues from ischemic damage. The study confirms neuroprotective potential of Estetrol in combating cerebral ischemia for the first time.

Besides Withanolide A and Estetrol, this study also explores neuroprotective ability of Prolactin in *in-vivo* global cerebral ischemia model. It was observed that Prolactin was able to significantly restore physiological parameters like blood pressure, heart rate and cerebral blood flow in ischemia induced rats. Prolactin post-treatment was able to reinstate the increased levels of neurotransmitters and elevated levels of cerebral calcium and nitrate levels along with

reduction of cerebral edema and infarction. Apoptotic and necrotic cell death in cortical region also decreased due to Prolactin post-treatment.

Thus, the present study infers the neuroprotective ability of three compounds, Withanolide A, Estetrol and Prolactin, against global cerebral ischemia. The efficient neuroprotection exhibited by these compounds in global cerebral ischemia model establishes them as potential choices for neurotherapeutics design in the future.

- **FUTURE WORK**

  i. Since Withanolide A showed ameliorating effect on cell death induced by global cerebral ischemia, further investigations are to be performed regarding its effect on *bcl2/bax* pathway, which reportedly contributes in apoptotic cell death pathway of cerebral ischemia.

  ii. The study shows Estetrol is able exert neuroprotection in global cerebral ischemic condition, further study is required regarding its mode of action. Since Estradiol is known to exert neuroprotection via Estrogen receptors α and β. Further investigation is to be done if Estetrol also have a similar mode of neuroprotective action and the mechanism of interaction of Estetrol with ERs is to be studied further.

  iii. It is predicted that Estetrol might inhibit RIPK1. Hence, further analysis of the ability of Estetrol in RIPK1 inhibition is to be done using *in-vitro* assays. Further comparison of RIPK1 inhibition ability of Estetrol and Necrostatins are also to be preformed, so that Estetrol might be designed as a future neurotherapeutics.

iv. It was reported that Prolactin reduced calcium overload in hippocampal neuronal cells subjected to glutamate excitotoxicity. In accordance to the reported study, the present study reveals that Prolactin post-treatment restores the calcium levels which increased due to induction of global cerebral ischemia. Hence, mechanism of Prolactin in restoring calcium concentration in ischemic condition is to be studied further.

v. Also, a furthet study is to be done for studying the synergistic effect of Estetrol and Prolactin in global cerebral ischemia.