PREFACE

This thesis is submitted for the degree of Doctor of Philosophy at Indian Institute of Technology (Banaras Hindu University), Varanasi. The research described herein was conducted under the supervision of Prof. Ranjana Patnaik in the School of Biomedical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi between July 2014 and February 2019.

This work is to best of my knowledge original, except where acknowledgements and references are made to previous work. Neither this, nor substantially similar thesis has been or is being submitted for any other degree, diploma or other qualification at any other university.

Cerebral ischemia, a global or focal deficiency of blood circulation to the brain, can arise through a complex cascade of events leading to neuronal lesions. Ischemic stroke is one of the leading causes of mortality and morbidity worldwide. The treatment for stroke in the acute phase involves use of thrombolysis, antiplatelet, anticoagulants and neuroprotecting agents. Thrombolysis using tissue plasminogen activator (tPA) or mechanical thrombectomy is widely accepted for the treatment of ischemic stroke. Both methods have a limited time of therapeutic window. Till date, rtPA is the only FDA approved drug available for treatment of acute ischemic stroke. Therefore, much interest is generated to use a neuroprotective agent that can halt the multiple pathway mediating neuronal damage or enhance the therapeutic windows of thrombolytic therapy. Several synthetic drugs has been reported as a neuroprotective agents to combat the ischemic condition but remain untranslated clinically due to adverse side effects and toxicity. Hence, use of active phytochemicals have attracted significant attention as an alternative to synthetic drugs.

The objective of the present thesis was to understand the pathophysiological changes of global ischemic stroke as well as its neuroprotection by chlorogenic acid-a major phytochemical present in green coffee and *Withania somnifera.* The chlorogenic acid was identified as a potent inhibitor for several molecular mediators of neuronal dysfunction using molecular docking simulation which is extensively discussed in chapter 3. Previous study demonstrated that chlorogenic acid likely enter to the brain by crossing BBB using a BBB in vitro model. Therefore, a study was performed to evaluate the brain penetration efficiency of this molecule using in vivo rat model which is demonstrated in chapter 4. Several methods for the determination of nitrate level in the brain have been reported but accuracy, precision, reproducibility and unavailability of instruments impose an obstacle for the estimation of the same. Therefore, a simple, rapid and reproducible method is developed for the determination of nitrate in brain tissue using HPLC-UV which is described in chapter 5. It is well known that impedance of rat brain is increased during ischemia and reversed after reperfusion but exact mechanism behind these changes are not fully understood. A study was designed to understand the changes of brain impedance with changes of electrolytes concentration during ischemia-reperfusion and presented in chapter 6. Due to controversies associated with 2 vessel occlusion rat model of cerebral ischemia the author has felt for a long time that a reproducible rat model of cerebral ischemia using hypotension coupled with two vessel occlusion should be developed without need of blood withdrawing procedure. Chapter 7 demonstrates the procedure for induction of hypotension using vasodilator drug followed by ischemia. The molecular docking study predicted chlorogenic acid as an inhibitor of NMDAR and nNOS. Therefore, an in vivo study is needed to validate the NMDAR and nNOS inhibition potential of CGA by keeping

ifenprodil and 7-nitroindazole as reference molecules. The chapter 7 demonstrates the neuroprotective effect of CGA in ischemic rat model.

The present study will be beneficial for the researchers/academicians/clinicians working in the area of neuroprotection, ischemic stroke and chlorogenic acid.

Part of this work has been presented in the following publications:

1. **Kumar G**, Paliwal P, Mukherjee S, Patnaik N, Krishnamurthy S, Patnaik R. Pharmacokinetics and brain penetration study of chlorogenic acid in rats. Xenobiotica. 2018 Mar 7:1-7. DOI:10.1080/00498254.2018.1445882
2. **Kumar G**, Paliwal P, Patnaik R. *Withania Somnifera* phytochemicals confer neuroprotection by inhibition of the catalytic domain of human matrix metalloproteinase-9. *Letters in Drug Design & Discovery*. 2017 Jan 1;14(6):718-26.
3. **Kumar G**, Paliwal P, Patnaik N, Patnaik R. *Withania Somnifera* phytochemicals confer neuroprotection by selective inhibition of nNos: An in silico study to search potent and selective inhibitors for human nNOS. *Journal of Theoretical and Computational Chemistry*. 2017: doi.org/10.1142/S0219633617500420
4. **Kumar G**, Patnaik R. Inhibition of Gelatinases (MMP-2 and MMP-9) by *Withania Somnifera* Phytochemicals Confers Neuroprotection in Stroke: An *In Silico* Analysis. *Interdisciplinary Sciences: Computational Life Sciences*. 2017 May 9:1-2.doi: 10.1007/s12539-017-0231-x
5. **Kumar G**, Patnaik R. Exploring neuroprotective potential of *Withania Somnifera* phytochemicals by inhibition of GluN2B-containing NMDA receptors: An in silico study. *Medical Hypotheses*. 2016 Jul 31; 92:35-43.