Summary and Conclusion
5. SUMMARY AND CONCLUSION

Neurodegenerative disorders are the growing socio-economic problem in many countries. Currently, many neurodegenerative disorders remain poorly understood with few known effective treatments, making research in this area a high priority. Huntington’s disease (HD) is an inherited neurodegenerative disorder characterized by progressive loss of neurons in the striatum. 3-Nitropropionic acid (3-NP) reproduces the brain lesions in laboratory animals as observed in HD patients. The mechanism by which 3-NP induces neurodegeneration involves mitochondrial membrane depolarization, energy depletion, oxidative stress, neuroinflammation and apoptosis. Recently, researchers have made considerable efforts on searching natural antioxidants in particular, plant derived polyphenolic compounds with neuroprotective potential for the treatment of neurodegenerative diseases. Naringin, a dietary flavonoid commonly found in citrus fruits exhibits diverse biological and pharmacological properties. The aim of this study is to elucidate the neuroprotective effect of naringin on 3-NP-induced neurodegeneration. To achieve this goal, a wide variety of different techniques from diverse disciplines including behavioral, biochemical, histological and molecular approaches were utilized. The following studies were performed to prove the beneficial efficacy of naringin on 3-NP-induced neurodegeneration.

- 3-NP-induced rats showed significant decrease in body weight. Treatment with naringin protected the loss of body weight in 3-NP-induced rats.
Neurobehavioral studies reflect movement impairment in 3-NP-induced rats. Naringin treatment improved motor function in 3-NP-induced animals.

The activities of pathophysiological markers like LDH and ALP were reduced upon treatment with naringin in 3-NP-induced rats.

Naringin protected the tissue damage by reducing the levels of \( \cdot \)OH, \( \text{H}_2\text{O}_2 \), MDA and protein carbonyl, which were elevated in 3-NP-induced rats.

Administration of naringin ameliorated the activities of enzymatic antioxidants and the levels of non enzymatic antioxidants to normal levels in 3-NP-induced animals.

The activities of Succinate: ubiquinone oxidoreductase, Ubiquinol: ferrocytochrome c oxidoreductase, Ferrocytochrome c: oxygen oxidoreductase (complex II, III and IV) were decreased in 3-NP-induced animals. The activities of these complexes were ameliorated upon naringin treatment.

The activities of ATPases (\( \text{Na}^+\text{K}^+\)-ATPase, \( \text{Mg}^{2+}\)-ATPase and \( \text{Ca}^{2+}\)-ATPase) were decreased in 3-NP-induced rats, whereas naringin augmented the activities of these enzymes.

Administration of naringin ameliorated the AChE activity in 3-NP-induced rats, which had decreased activity than control group of rats.

Histological analyses in striatum revealed the presence of darkly stained nonviable neurons, damaged cells with condensed and pyknotic nuclei. Naringin treatment resulted in modulation of these abnormalities in the striatal histopathology near to normal with the
presence of lesser pyknotic nuclei denoted the protective efficacy of naringin.

- Ultrastructural changes of striatum in 3-NP-induced rats showed swollen mitochondria with loss and disorganized cristae. Treatment with naringin showed moderately disorganized cristae. Further, the degree of chromosomal condensation and marginization induced by 3-NP was considerably reduced in rats treated with naringin.

- The protein and mRNA expressions of MMP2 and MMP9 were elevated by 3-NP. Naringin showed its protection by reducing the expressions of these MMP’s in striatum of 3-NP-induced rats

- 3-NP-induced rats showed decreased expressions of TIMP-1 and TIMP-2. The reduction was significantly ameliorated upon naringin treatment.

- Naringin showed its anti-inflammatory effect by reducing the expressions of inflammatory markers like NF-κB, TNF-α, COX-2, GFAP, iNOS and nNOS in striatum of 3-NP-induced rats.

- Increased expressions of Bax and Bad along with decrease in the expression of Bcl-2 were observed in 3-NP-induced animals. Treatment with naringin significantly altered the expressions of these apoptotic markers near to normal. Further naringin decreases the expression of cytosolic cytochrome c and inhibits caspase 3 activation.

- Administration of naringin increased the expressions of Hsp27 and Hsp70 in 3-NP-induced rats and mitigates the pathogenesis of neurodegeneration.

- 3-NP-induced rats showed mild increase in nuclear Nrf2 expression when compared to control animals. Whereas naringin treatment
significantly increase the nuclear Nrf2 expression as compared with 3-NP-induced and control groups of rats, with relative decrease in cytosol indicating the activation of Nrf2.

- As similar to Nrf2 activation, naringin treated rats exhibited significant increase in mRNA expressions of NQO-1, HO-1, GST-P1 and γ-GCL, and confers protection in 3-NP-induced neurodegeneration.

- The mRNA expressions of BDNF and TrkB were diminished in rats exposed to 3-NP. The reduction was ameliorated upon naringin treatment.

- To elucidate the mechanism by which BDNF protects neurons from 3-NP-induced cell death, the activation of PI-3K pathway is examined by analyzing the changes in phosphorylation status of Akt. Naringin treatment augmented the expressions of PI-3K and p-Akt in 3-NP-induced rats, which had lowered expressions than control rats.

- After validating the protective role of naringin against 3-NP-induced neurodegeneration in vivo, the efficacy in PC12 cell line was tested to prove its neuroprotective effect in vitro and to study the convergence of BDNF/TrkB and Nrf2 signaling pathway.

- Elevated LDH release was observed in 3-NP-induced PC12 cells. Treatment with naringin decreased the LDH activity towards normal. The activities of enzymatic antioxidants and GSH level were ameliorated by naringin in 3-NP-induced cells.

- 3-NP caused an intense oxidative stress characterized by increase in LPO product and ROS. Administration of naringin renders protection to PC12 cells by reducing the levels of LPO and ROS accumulation.
Further, naringin ameliorates the mitochondrial membrane polarization in the cells exposed to 3-NP.

- 3-NP exposure induced the phosphotidyl serine exposure and altered mRNA expressions of Bcl-2 and Bax. Naringin showed its anti-apoptotic effect by decreasing the phosphotidyl serine exposure and by modulating the expressions of Bcl-2 and Bax to normal level.

- Naringin renders protection against 3-NP-induced neurodegeneration through increased nuclear accumulation of Nrf2 and subsequent phase II gene expression.

- To determine the involvement of PI-3K/Akt in naringin-driven activation of Nrf2, LY294002, the pharmacological inhibitor of PI-3K/Akt signaling was used. Naringin-induced Nrf2 nuclear translocation and its target genes NQO-1 and HO-1 expressions were effectively reduced by LY294002 suggesting that PI-3K is vital in the activation of Nrf2 in PC12 cells.

This study provides evidence that naringin exhibits neuroprotective effect on 3-NP-induced neurodegeneration through its antioxidant, anti-inflammatory and antiapoptotic properties. Further, naringin upregulates the nuclear accumulation of Nrf2 and enhances the expressions of phase II and antioxidants genes. The action of naringin is also associated with the activation of BDNF/TrkB signaling and its downstream sequences PI-3K and Akt. Further, naringin mediated nuclear translocation of Nrf2 occurs via the activation of BDNF/TrkB pathway. This study demonstrates the possible mechanisms underlying the protective role of naringin against 3-NP-induced neurodegeneration and suggests that naringin could act as a potent neuroprotective agent.