8. SUMMARY, CONCLUSION AND FUTURE PERSPECTIVES

Parkinson's disease is a progressive neurodegenerative disorder, characterized by motor and other behavioural impairment. The primary pathological hallmark of PD is the loss of nigrostriatal dopamine (DA), driven by inflammation, oxidative stress and mitochondrial impairment. As evident, till date no satisfactory therapeutic modalities are available for the treatment and management of PD. The current hypothesis concerning the pathogenesis of idiopathic PD indicates that selective oxidative stress and inflammatory cascades, that expresses itself with compatible biochemical changes in mid brain and striatum. Therefore, putative drug candidates who offer free radical-scavenging, anti-inflammatory and immuno-modulatory properties are potential therapeutic agents for the treatment and management of PD. The major objective of the present research work was to investigate neuroprotective effect of withanolides and curcuminoids which can protect nigrostriatal dopaminergic neurons against MPTP-induced cytotoxicity. The neurotoxin, MPTP mimics and produces cardinal features of PD and a reliable experimental model.

In this context, the present comprehensive research work provides scientific evidence about the neuroprotective effect of withanolides and curcuminoids against MPTP induced dopaminergic degeneration. In in-vitro studies both withanolides and curcuminoids exhibited antioxidant and NO inhibition activity in dose dependent manner however, in MAO-B inhibition activity and chelating properties curcuminoids has exerted significant effect as compared with corresponding standards.

Oral administration of withanolides in MPTP-induced C57Bl/6 mice modulated catecholaminergic neurotransmission particularly, DA and NE, which was also reflected in behavioral activity where significant improvement were observed in rota-rod, open field activity
and tail suspension test performances. Among other Parkinsonian marker parameters; significant up-regulation was observed in tyrosine hydroxylase (TH) expression whereas no marked effect was found over MAO-B level in withanolides treated mice. Henceforth, the present findings suggested that withanolides administration did not interfere in the model development.

The present findings demonstrated that neuroprotective activity of withanolides against MPTP-induced dopaminergic degeneration was attributed in-part due to the amelioration of specific mitochondrial complexes viz complex I and IV activity which overall modulated the oxidative phosphorylation and thus prevented depletion of ATP content. In addition, amelioration of mitochondrial complexes also reduced the generation of reactive oxygen species which otherwise targets both mitochondrial complexes as well as dopaminergic neurons. In the present study pre-treatment with withanolides restored the endogenous antioxidant enzymes particularly glutathione and superoxide dismutase and reduced protein carbonyl content which was altered in diseased condition. Moreover, withanolides administration also prevented the membrane peroxidation as indicated by reduced LPO and LHP content in MPTP-induced mice brain. Based upon the correlation findings it was hypothesized that the modulation of SOD and LHP activity was attributed for the improvements in neurobehavioral paradigms in withanolides treated MPTP mice.

Among the inflammation mediated dopaminergic degeneration in MPTP model of PD, pre-treatment with withanolides significantly suppressed the pro-inflammatory cytokines particularly IL-1β, TNF-α and GFAP protein expression. Further, withanolides administration was observed to down-regulate iNOS and nNOS protein expression with concomitant inhibition of total nitrite content. From the present findings, it was speculated that the neuroprotective effect of withanolides was attributed in-part to its significant immunomodulatory property.
Correspondingly, curcuminoids have demonstrated neuroprotective effect through modulating the DA and 5-HT content and exerted significant effect over motor coordination, depression and total gross behavioural activity in treated group as compared with MPTP alone group. Furthermore, curcuminoids administration exerted significant protection over tyrosine hydroxylase content without affecting the MAO-B content in the treated groups.

The present study has observed that pre-treatment with curcuminoids exerted significant protection over the mitochondrial complexes, particularly Complex I by inhibiting the binding of MPP⁺ to the complex as well as scavenging the reactive oxygen and nitrogen species. However activity of other mitochondrial complexes was also found to be alleviated on curcuminoids treatment in MPTP model of PD. In one of the correlation analysis it was suggested that improvement in complex II activity leads to the significant anti-depressant effect on curcuminoids administration. Moreover, present data suggested that curcuminoids exhibited potent antioxidant activity as indicated by the restoration of endogenous antioxidant enzymes \( \text{GSH, GPx, SOD} \) and catalase as well as attenuation of LPO, LHP and carbonyl content in the treated group. In correlation studies a direct association was observed between the reduction of oxidative stress and elevation of behavioural functioning curcuminoids administration.

Moreover, pre-treatment with curcuminoids protected dopaminergic neurons by attenuating IL-1β, TNF-α and GFAP expression and mitigates iNOS expression. Furthermore, correlation finding has supported that curcuminoids administration improved the motor coordination by attenuating TNF-α expression.

Taken together the present findings demonstrated the pleiotropic mechanism of action of withanolides and curcuminoids against MPTP induced dopaminergic degeneration. It is apparent that withanolides might have acted as potent neuroimmune modulator and thus functionally compensating for the loss of dopaminergic neurons whereas curcuminoids primarily attenuated
oxidative stress markers and associated mitochondrial impairment which might have protected
the progression of remaining dopaminergic neurons.

It is evident that PD is a syndrome rather a disease, which involves intricate multiple
etiological factors responsible for the progressive neurodegeneration. Thus the present findings
supported the usage of combination drugs which exert preventive and pleiotropic mode of action
to overcome intricate cellular events culminating to neurodegeneration. However, the present
preclinical findings warrants extensive research, particularly individual components efficacy to
acclaim the drug-drug interaction as well as to validate the pleiotropic, synergism and antagonism
mode of action of the individual components of the tested drug candidate to support the present
findings. Thus, the present research work suggested that both withanolides and curcuminoids
possessed immense potential as a putative drug candidate and warrants further research to
extrapolate preclinical findings into clinical studies for better treatment and management of PD.