CHAPTER - V

SUMMARY AND CONCLUSION

The plant *Mucuna* belongs to the family *Leguminosae* is indigenous to tropical countries like India and West Indies. Traditionally, in Ayurvedic system of medicine, *Mucuna* has been used in the treatment of Parkinson’s disease and other neurodegenerative diseases. L-Dopa based combination therapies are most preferred due to their efficiency in the control of Parkinson’s disease. But on the other hand the global demand for L-Dopa is high but its production is very low. Thus it is crucial to develop alternate strategies which are economically viable for the commercial production of L-Dopa.

In this connection, the envisaged present study was conducted on elicitor mediated enhancement of L-Dopa and other phenolics and further to prove the neuroprotective of the same. Primary callus were established from the leaf explants of *Mucuna* and used for secondary callus production. Elicitated callus was studied further for experimental analysis. Media components were optimized to establish callus by manipulating the concentration of macro, micro nutrients, pH, sucrose and auxins and cytokinins (0.1mg to 10mg L⁻¹). The growth of the callus was measured by recording the fresh weight and dry weight. The secondary callus thus obtained by inoculating in a MS solid medium supplemented with 2,4-D 4.52 µM ,BA 2.22µM and 2ip 4.92µM.

Friable, soft and nodular callus of *Mucuna* was induced from leaf segments of *in vitro*-grown seedlings on modified MS medium supplemented with 2,4-D 4.52 µM ,BA 2.22µM and 2ip 4.92µM.

Media optimization was carried out with in D- optimality criteria method. Most effective combination of the salt groups was obtained at run 9. The fresh weight at the optimized point was seen to be 245g L⁻¹. The optimization procedure was repeated on three subcultures each of the different combination of media salts. The levels of sucrose, pH macro/micro nutrients were also optimized.
This is a new approach to optimize the media for the micro propagation of *Mucuna*. This novel method provides a non time consuming, low cost method of optimization of callus growth. The novelty of this study is further enhanced by the usage of response surface modelling. The most effective treatment points were the 5th and 11th points, with maximum fresh weight (197g L\(^{-1}\)) and maximum dry weight (17.34g L\(^{-1}\)) in both. The optimization was repeated on three subcultures at each treatment point. These same methods may also be adopted for other plants with a basic morphological or genetic similarity to the current species.

To enhance the production of L-Dopa content, the callus is treated with elicitors viz., SA and MeJA individually or in combination showed each of these resulted in an enhancement in the L-Dopa production. The enhanced L-Dopa levels were found to be correlative with higher Ca\(^{2+}\)ATPase activity suggesting the involvement of calcium in during the elicitation process. The endogenous titres of *A.niger* were found to correlate with the growth of the callus. However, on comparison, the performance on the enhancement of L-Dopa, *A.niger* and MeJA were brought better results than SA. But both of these treatments resulted in an enhancement of the L-Dopa content, which would suggest that the L-Dopa production in *Mucuna* cell cultures is significantly influenced by *A.niger*.

The enhancement of L-Dopa was proved by calmodulin pathway in the calcium was observed to have had a more major role in the L-Dopa production than that of the elicitation mediated by other elicitors. These prove that L-Dopa elicitation occurs through the calcium –calmodulin pathway. It would be of paramount importance to know the amount of elicitation possible by the use of effective elicitors coupled with calcium signalling enhancers, which may additionally improve L-Dopa production. Thus it was performed and from the results, it was observed that the addition of the elicitor resulted in an increase in the L-Dopa production in the callus cultures of *Mucuna* on the 9th day of culture. The ionophore, in combination with the elicitor further elevated the levels of L-Dopa which was then lowered by the addition of calcium-channel blockers on the 21st day, which strongly suggesting the involvement of calcium channel during elicitor-mediated enhancement of L-Dopa production.
Neuroprotective effect of crude elicited callus culture *Mucuna* was studied against N27 dopaminergic neuronal cell lines has also been performed and reported. The effects of the extract of *Mucuna* seed and callus extracts on these cell lines against MPP\(^+\) toxin are seen to be excellent.

MPTP is a potent neurotoxin that causes selective loss of dopaminergic neurons and causing Parkinson’s disease like symptoms in human as well as in animal models on MPTP exposure in *in vitro* caused a marked concentration-dependent induction of oxidative stress in homogenates and synaptosomes to some regions of the brain of swiss albino mice. Further to establish the neuroprotective effects of *Mucuna* against Parkinson’s disease, *in vivo* studies were carried out on Swiss albino mice. *Mucuna* aqueous extract exhibited a concentration dependent free radical scavenging activity in various chemical systems suggesting an excellent antioxidant potential from *Mucuna* components.

*Mucuna* seed and callus extracts in combination attenuated the neurotoxicity due to MPTP in experimental mice. The neuroprotective effect of *Mucuna* extract combination was observed in the experimental PD mice. Moreover, it improves the neurochemical levels, antioxidant status and behavior patterns significantly. These results suggested that the anti- Parkinsonic effects of *Mucuna* are very high and these findings also provided a therapeutic basis for the clinical application of this drug pair.

MPTP has been shown to induce oxidative stress, decrease tyrosine hydroxylase activity, increase inflammation and mitochondrial dysfunction leading to dopamine depletion and increased GABA in the striatum. Our result suggests the *Mucuna* combination has a potentially important role in the management of patients with neurodegenerative disease. The results of this study also clearly establish the ameliorating effects of *Mucuna* on MPTP induced Parkinsonism. In the present study, we investigated the *Mucuna* extract as compared to some of the modern medicines used in the treatment of Parkinson’s disease and these studies have revealed heightened natural antioxidant and anti-inflammatory actions of these molecules which make them a potential neuroprotective agent against dopaminergic neurotoxicity.
Statistical nutrient optimization was done to optimize both biomass and L-Dopa production in *Mucuna* callus cultures. D-Optimality criteria demonstrated significant effect of macro, micro nutrient level in the culture media. Mathematical model equations with respect to biomass and L-Dopa were generated to describe the complex nutrient interactions. The equations were used to develop contour plots for nutrient effects. Analysis of contour plots for respect to biomass accumulation yielded the optimum nutrient media. Experiment conducted with the optimized media composition pH and Sucrose resulted in significant biomass accumulation.

From this study, it may also be concluded that it is possible to increase the content of L-Dopa in *Mucuna* by optimizing and regulating the plant growth regulators in the tissue culture media. It is also possible to eliminate the shortage of *Mucuna* plant and satisfy the demand for this plant in pharmaceutical industries as a source for L-Dopa using micro propagation. The enhancement of L-Dopa has been achieved in calli by the use of fungal elicitors, precursor feeding and abiotic elicitors. The stimulatory effect of MeJA and *A. niger* on the production of secondary metabolites is well documented.

Thus, the efficacy of *Mucuna* callus with enhanced L-Dopa content as a neuroprotective agent are clearly established and further commercial exploitation of the same may be possible in the near future.