8. SUMMARY AND CONCLUSION

The present study was aimed to target a nondopaminergic pathway by using liposomal formulation, which may be a novel therapeutic treatment for Parkinson’s disease. The two different animal models were used to induce the Parkinson’s disease viz. 6-OHDA and MPTP to evaluate the mechanistic action of selected drug molecules. From extensive literature survey and scientific studies the histamine H3 receptor (H3R) antagonist Conessine and c-Jun N-terminal kinase-3 (JNK-3) inhibitor 1,9-Pyrazoloanthrone (1,9-P) were selected as drug candidate to in present study.

To check the potency of selected drug molecules we performed in silico docking studies by using histamine receptors (H-1, H-2, H-3) and JNK kinases (JNK-1, JNK-2, JNK-3), and enzyme kinetic activity of 1,9-P on different MAP kinase enzymes. Results showed that, both selected molecules Conessine and 1,9-P have higher affinity towards the H-3 receptor and JNK-3 enzyme respectively.

In present study, we successfully proposed the Conessine and 1,9-P loaded liposomal formulation to cross the BBB and target the brain by minimum or no peripheral side effects. The developed formulations were evaluated for various physiochemical characteristics like particle size, zeta potential, entrapment efficiency and polydispersity index (PDI). The surface morphology of liposomes was confirmed with SEM and shows the spherical morphology of liposomes that helped in sustain release action and stability of formulations. The compatibility and crystalline behavior of Conessine and 1,9-P with excipients were evaluated by using DSC and FTIR. In vitro drug release studies by dialysis bag method had showed initial burst release of drug followed by sustain release over a period of 24 h.

The pharmacokinetic and biodistribution study of prepared liposomes in comparison with pure drug were performed on Wistar rats. This study showed better pharmacokinetic parameters for developed liposomes than pure drug, the order of the area under curve was found to be kidney> liver> brain> lungs> spleen> heart. The liposomes of Conessine and 1,9-P were rapidly taken up into brain and showed a good brain concentration after 2.0 h; sustenance up to 4.0 h was achieved which is better than pure drug solution.

To assess the cytotoxic of prepared liposome MTT assay was performed using SH-SY5Y neuronal cells. Pre-treatment with 1,9-P and Conessine liposomes at 1-100 µg/mL for 18 h caused no cell toxicity. The protective effects of selected nanoformulation on 6-OHDA and MPP⁺ induced neurotoxicity shows cells pre-treated with 1,9-P liposomal formulation
Summary and Conclusion

Significantly protected the cells up to 85.76% and 80.78% for 6-OHDA and MPP⁺. AO/EB and Hoechst 33342 staining assay were confirmed the anti-apoptotic/neuroprotective effect of developed liposomes. Furthermore, DNA fragmentation assay with anti-apoptotic and pro-apoptotic markers estimation were performed using western blotting, which showed significantly increased anti-apoptotic marker Bcl2 with reduced DNA fragmentation and pro-apoptotic markets viz. Bax, caspase 3, cytochrome-c, p-p 38 and p-JNK in treatment groups.

The induction of Parkinsonism was done by cerebrospinal injection of 6-OHDA and MPTP and confirmed by apomorphine HCL challenge. The various behavioral parameters were performed to evaluate anti-Parkinson activity. The striatal dopamine level evaluated by HPLC along with molecular estimation of apoptotic and anti-apoptotic protein estimation support the anti-parkinson's activity of developed formulations. The iron degeneration estimation by perl's DAB staining along with histopathology of brain striatum demonstrates the reversal to histology with regeneration of neurons which gives additional cushion to above findings.

The study concludes that the 6-OHDA has more potential to induce idiopathic early stage of PD than MPTP. Also, study demonstrate the liposomes of 1,9-P and Conessine possesses neuroprotective and dopamine enhancing potential respectively. The important outcome of study is combination therapy of selected drugs shows better and effective action than single drug treatment. The current drug therapy can lay a new path to treat the Parkinson’s disease by targeting alternative pathway than dopaminergic system.

Future Work:

1. The long term toxicity of developed drugs along with therapeutic drug monitoring (TDM) needs to be performed to find out the adverse effects of drug candidate.
2. Preclinical studies in higher model of animals followed by clinical study of developed formulations.