Preface

The research work of the thesis entitled "Glucocerebrosidase chaperone as a treatment strategy for Parkinson's disease (PD)" identified the possible novel glucocerebrosidase (GCase) chaperone for the potential treatment of PD. PD is a result of nigral dopaminergic neuronal degeneration and toxic build-up of the oligomeric form of α-synuclein. Currently, PD affects millions of people around the world and is incurable to date. Hence, there is a need if new treatment strategy along with suitable experimental models to screen and evaluate new therapeutic compounds. New treatment strategy for PD by stabilization of Glucocerebrosidase (GCase) enzyme by chaperones is of particular interest. However, it is worth noting that the current research primarily relies on genetic models of GCase, which are applicable to only a small percentage of PD cases. These genetic cases account for approximately 2-5% of all Parkinson's patients, while the majority, around 90-95%, are classified as sporadic cases. Wild-type rat is a widely used model for the PD at the initial phases of drug discovery but unfortunately, the commercial rGCase and its 3D structure are unavailable. Hence, the lack of an established wild-type GCase *3D model* and *in vitro* method may poses a challenge when it comes to screening any novel GCase chaperone. This could be a reason for the lack of potential chaperones in the preclinical and later on the clinical trial for PD. The process to select suitable chaperones with more protein stabilising potential through virtual screening during the drug discovery process is also unexplored. Even its precise mechanism in PD with GCase deficiency is not yet known. Thus, in this study, *in silico*, *in vitro* and *in-vivo* evaluations of a chaperon on rGCase were established. Computational drug discovery techniques were employed to

find new possible GCase chaperones with the disease-modifying potential to treat PD. By using *in silico* molecular docking and molecular dynamics simulation; the capacity of screened compounds to stabilize GCase was assessed, and its effectiveness was then verified *in vitro* (Structural stability assay). To examine their potential as GCase chaperones for the treatment of PD, cell lines were used. Finally, to assess its anti-PD potential with a comprehensive molecular mechanism in a 6-OHDA – induced PD model. To assess the motor abnormalities, we conducted a variety of behavioral tests. The protein expression of oligomeric α-synuclein and tyrosine hydroxylase (TH) was estimated. Neurochemical profile was measured by calculating striatal dopamine. Loss of nigral dopaminergic cells was examined by analysing TH-positive cells. The whole work has been compiled into six chapters. **Chapter 1** describes the introduction and significance of the present study. **Chapter 2** describes the rat Glucocerebrosidase homology modelling and mechanism of chaperone **Chapter 3** describes the virtual screening, molecular docking, and molecular dynamics techniques to identify and assess a promising glucocerebrosidase chaperones for potential treatment of PD **Chapter 4** describes the oral acute toxicity study of a selected chaperone in rats **Chapter 5** describes the neuroprotective effect of a top screened chaperone with the molecular mechanism in a 6-OHDA-induced rat PD Model. **Chapter 6** describes the HPLC method for quantification of GCase chaperone in rat plasma and brain. **Chapter 7** summarizes the entire study completed with its essential outcomes.

By addressing this gap in our knowledge and establishing 3D models and GCase-chaperone interaction mechanism, scientists will be better equipped to explore the role of GCase activity in sporadic PD cases. Such advancements will contribute to a more comprehensive understanding of the disease mechanisms and potentially open up new avenues for therapeutic interventions targeting GCase and related pathways.