Parkinson’s disease (PD) is a most common neurodegenerative disease. The main pathological hallmark of this disorder is a selective loss of dopamine-producing neurons in the substantia nigra, which results in a drastic depletion of dopamine in the striatum. Another pathological characteristic of PD is the presence of eosinophilic, cytoplasmic inclusions of fibrillar, misfolded proteins, termed Lewy bodies (LB), in the affected brain areas. PD affects approximately 1% of population which is above 65-years. 90-95% of the cases are of sporadic origin. Although PD is considered as motor dysfunction disease but non-motor symptoms associated with disease precedes the motor symptoms which are used as early diagnosis marker. The common symptoms are postural changes, gait disorder, muscle rigidity, Akinesia, dyskinesia, Sleep disturbance, Depression, Fear or anxiety, Memory difficulties and slowed thinking, Sexual dysfunction, Urinary problems, Fatigue and aching, and Compulsive behavior.

The common therapeutic approaches includes dopamine replacement therapy by administration of L-dopa, Monoamine oxidase – B (MAO-B) inhibitors which prevent the degradation of dopamine, COMT inhibitors as Catechol-O-methyltransferase (COMT) inactivates dopamine, dopamine agonists to stimulate the dopamine receptor and anticholinergics to prevent the exaggerated motor effects of acetylcholine, other treatments involves surgical interventions in form of Deep brain stimulation, gene therapy and stem cell implantation. However, all these therapeutic agents have side effects leading to the development of better therapeutic agent with higher efficacy and lower side effect. The symptoms of PD are majorly the result of apparent increase in acetylcholine level in the brain due to the decrease in the inhibitory neurotransmitter GABA by the neurodegeneration of dopaminergic neurons. A variety of factors are responsible for the degeneration of dopaminergic neurons resulting in PD like neuronal vulnerability, genetic factor, environmental factors, neuroinflammation and aging. Nigral dopaminergic neurons are particularly vulnerable to oxidative stress because the metabolites of dopamine can act as endogenous toxins. Dopamine auto-oxidizes at normal pH into toxic dopamine-quinone species, O$_2^-$ radicals and H$_2$O$_2$. Superoxide can be converted to H$_2$O$_2$ by super oxide dismutase, or into labile ONOO$^-$ radicals in the presence of Nitric Oxide (NO). H$_2$O$_2$ can be broken down into cytotoxic hydroxyl radicals in a chain of iron-mediated reactions. In PD, nigral cells seem to be under a heightened state of oxidative stress, as evidenced by the presence
of high level of by-products of lipid, protein and DNA oxidation. There are various familial forms of PD, including those linked to mutations in genes like, α-synuclein (PARK1), parkin (PARK2), DJ-1 (PARK7), and PTEN (phosphatase and tensin homolog deleted on chromosome 10)-induced kinase 1 (PINK1, also known as PARK6) which suggest the important role of ubiquitin proteasomal system in the molecular pathology of PD. Environmental Toxins like weedicides, insecticide (dieldrin, lindane, parquet, and rotenone), organic solvents, metals (lead, manganese, iron, copper, and zinc), infectious agents, dietary intake of dairy product and synthetic heroin (MPTP 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydro pyridine) causes neuronal cells death both in vitro and in vivo models of PD. Piles of evidences suggest that NO overproduction also plays a key role in the pathogenesis of PD. NO and its toxic metabolite ONOO- impair the mitochondrial respiratory chain by affecting the function of complex I, complex II, and complex IV, leading to energy failure and ultimately cell death. Nitroative Oxidative Stress is of two types: “Nitrosation” means covalent incorporation of a NO diatomic group to another group and other is “Nitrosylation” means addition of NO to a metal by a complex (or coordinating) bond. Addition of Nitric Oxide to thiols of cysteine residues of the proteins is called S-nitrosylation, Its formation is more favored in more ionisable cysteine, such as those in which the thiolate anion can be stabilized by acid-base interaction with neighboring groups, either belonging to adjacent residues in the primary sequence or just in the proximity in the three dimensional structure. Thus pKa of different cysteines residues play important role in determining the occurrence of S-nitrosylation. protein nitration leads to protein aggregation (α-synuclein) which are highly toxic to neurons and can promote neurodegeneration. In addition to inducing protein aggregation, recent studies show that nitrosative stress can also compromise a number of neuroprotective pathways by modifying activities of certain proteins through S-nitrosylation which is otherwise an important post translational modification involving in the cell signalling. Due to the toxic effect of high levels of Nitric oxide synthesized by nitric oxide synthase NOS, it is emerging as new therapeutic target in many diseases. NOS occur in three isoforms: nNOS (expressed in central and peripheral neurons), eNOS (expressed in endothelial tissues), and iNOS (expressed in macrophages). Among these nNOS and eNOS are constitutively
expressed in mammalian cells and synthesize NO in response to increases in intracellular calcium levels while iNOS is independent to calcium level and is inducible in nature in response to external stimuli. All the isoforms shares structural homology with each other by possessing oxygenase and reductase domain for the synthesis of NO but nNOS contains a PDZ domain (where PDZ stands for \textit{PSD-95 discs large} \textit{ZO-1} homology domain, and where PSD-95 stands for post synaptic density protein 95) that targets nNOS to synaptic sites in brain and skeletal muscle. The PDZ domain mediates the membrane association of nNOS in neurons through protein-protein interaction. The functions of nNOS include synaptic plasticity in the central nervous system (CNS), central regulation of blood pressure, smooth muscle relaxation, and vasodilatation via peripheral nitrergic nerves. However, due to the involvement of NOS enzyme in pathology of neuronal diseases, it became important to inhibit the activity of NOS enzyme to prevent and minimize the damage caused by excess formation of NO resulting in death of neuronal cells. In the pursuit to synthesize novel nNOS inhibitors, better understanding of the nNOS structure by cloning of nNOS and \textit{in vitro} cell based screening method was required, previously reported nNOS and \textit{in vitro} screening methods were either tedious method or have limitations of radioactive exposure and lesser selectivity towards between nitric oxide and reactive oxygen species respectively, which led to search for new method more selective to nitric oxide. 7-Nitroindazole (7-NI), nNOS inhibitor, has been shown to have neuroprotective effect in the 6-OHDA induced animal model of PD. However, these reports are based on unilateral model of male rat/mice and the mechanism of action of 7-NI is poorly understood which creates gaps in the knowledge. Present study points the following lacuna in the literature:

1. Easy purification and expression of nNOS protein in bacterial system and development of new screening method.
2. Bilateral rat model induced by 6-OHDA mimics the human pathology of PD but the neuroprotective effect of 7-NI in the females rodent model of PD is unknown.
3. Effect of 7-NI at protein level is poorly understood, further studies are required.
4. The effects of nNOS mediated by protein-protein interaction via its PDZ domain are not elaborated further studies are desired.
In an attempt to fill the above lacunas and to study the role of nNOS in the molecular pathology of PD, the present study was undertaken, which is divided in five chapters: chapter 1 Introduction and review of literature on the subject, chapter 2 The cloning of nNOS to develop in vitro screening method and functional studies for novel nNOS inhibitor, chapter 3 The development and validation of a bilateral 6-OHDA induced female rat model of PD, chapter 4 Comparison of NO mediated alterations in the total protein expression with S-nitrosylated protein expression profile in 6-OHDA-induced rat models and chapter 5. Identification of novel interacting protein partner/s of nNOS by the virtue of its PDZ domain to illustrate its role in molecular pathology PD and target novel proteins for better therapeutics.

Chapter 1 comprises the introduction to PD, its pathophysiology, factors responsible, current therapeutics and role of nitric oxide in PD is thoroughly described with the reference to known literature.

Chapter 2 describes the novel cloning of Rat nNOS for its easy purification from the bacterial system. Although rat nNOS have been previously clone and expressed in bacterial expression strains but the its purification was tedious as the cloning vector did not possess any affinity tag, in the present work rat nNOS has been recloned in a new expression vector which possess two affinity tags namely: Avi-tag (biotin tag) and His-tag (histidine tag) for the easy purification. The purified protein can be used for functional assays as well as for in vitro screening of nNOS inhibitor.

Chapter 3 describes the development of bilateral 6-OHDA induced female rat model of PD. In the present work to study the role of nNOS and to validate the nitrosative stress caused by 6-OHDA, 7-NI (inhibitor of nNOS) was used. Thirty five female rats were used for the present study and the animals were divided into four groups: Group 1: Control(n=8) The vehicle was administered surgically into the substantia nigra of 4 animals, and remaining 4 animals as untreated control. Group 2:7-NI(n=8) The animals were administered with 7-NI intraperitoneally(I.P) for three consecutive days. The 7-NI used was first dissolved in 0.005 % of DM SO and then mixed with ground. Group 3:6-OHDA(n=11)6-OHDA (10.5 µg) was administered in substantia nigra surgically. Group 4:6-OHDA+7-NI(n=8)The animals of this group were treated with
7-NI (30 mg/Kg body weight) for three consecutive days post surgery. The female rat were lesioned bilaterally with 10.5 µg per injection and animal models were validated by behavioural studies for emotional parameter (by forced swim test), cognitive parameter (olfactory discrimination test and elevated plus maze), and motor function test (by open field test and Rota rod test). Results indicates that 7-NI have neuroprotective activity against the 6-OHDA induced neurotoxicity. Forced swim test indicates that 7-NI possess anti-depressant activity as the 6-OHDA lesioned animals treated by 7-NI performed significantly better than the lesioned animals. Similar effect was observed in elevated plus maze which further supports the anti-depressant activity of 7-NI. Also, open field analysis suggests that 7-NI did not have any adverse effect on the spontaneous locomotion of the animals which is in accordance with the reports by previous workers. Rota rod test revealed the enhanced motor coordination by 7-NI. Neurochemical analysis through TH-immunohistopathology and dopamine concentration estimated by HPLC analysis shows the higher tyrosine hydroxylase (TH) positive neurons and higher level of dopamine in 7-NI treated group in comparison with 6-OHDA lesioned animals. The biochemical assays of glutathione estimation, lipid peroxidation, superoxide dismutase, and catalase also supports the anti-oxidant effect of 7-NI. Overall, 7-NI has neuroprotective effect in female bilateral rat model of PD induced by 6-OHDA.

Chapter 4 describes the NO mediated changes in the total protein expression level with the S-nitrosylation level of the total protein. It was achieved by analyzing the total protein expression profile with that of total S-nitrosylated proteins isolated from the animal models described in chapter 3. Both total protein samples and S-nitrosylated protein samples from each animal group were subjected to 2-dimensional gel electrophoresis and 2-dimensional in gel difference electrophoresis (2-DIGE) using Cy dyes respectively. The results revealed that 7-NI did not cause significant changes in the total protein expression levels but differentially changes the levels of S-nitrosylation among the animal treatment groups. Another significant observation was the changes in the S-nitrosylation level of Glyceraldehyde-3-phosphatdehydrogenase (GAPDH) alongwith differential expression in the animal treatment groups and its presence in the nuclear protein of the 6-OHDA and 6-OHDA+7NI
treatment group, which suggest the plausible mechanism for the 7-NI mediated neuroprotection by inhibiting the nuclear migration of GAPDH.

Chapter 5 attempts to find the novel nNOS interacting protein partners to mediate the protein-protein interaction by its PDZ domain to illustrate its role in molecular pathology PD and target novel proteins for better therapeutics. Protein-protein interactions are commonly studied either by co-immunoprecipitation or pull down assay but in present study both of these techniques were utilized in combination with in gel difference electrophoresis (2-DIGE). Results revealed rab GDP-dissociation inhibitor (rab GDI) beta and rab GTPase-activating protein 1-like proteins as the important interacting partners as both of these proteins are involved in nNOS mediated cell signaling.

In conclusion, the present work intended to study the role of nNOS in the molecular pathology of PD suggests the pathological role of nNOS due to its overproduction in 6-OHDA induced bilateral female rat animal models was significantly causing the neurotoxicity and it is further validated by the inhibition of nNOS by 7-NI which effectively reverse the toxic effects of NO in the 7-NI treated animal groups. The 7-NI exerts neuroprotection by acting as anti-oxidant, and anti-depressant not only at cellular level but at molecular level too as indicated by the decrease in the S-nitrosylation levels in the 7-NI treated animal groups.