CHAPTER 2
LITERATURE SURVEY

The pathological hallmarks of PD are the loss of neuromelanin containing dopaminergic neurons in SNpc brain regions, projecting to ST. The loss of these dopaminergic neurons produces classic gross neuropathological findings of SNpc depigmentation (Figure 2.1) (Marsden 1983).

![Image](Image)

**Figure 2.1.** Neuropathology of PD – loss of neuromelanin containing SNpc brain regions (Adapted from The New York Times, 2013)

Most features of PD tends to appear apparent only when the loss of dopaminergic cells occurs approximately at 50-60% in SNpc and 70-80% in ST brain regions (Ozansoy and Basak 2013). LBs pathology through α-syn aggregation has been a widespread cellular finding in PD. Various studies suggested that alterations in SNpc occurred after accumulation of LBs progressively in lower brainstem and olfactory system as well as throughout the body (Braak et al. 2003; Del Tredici and Braak 2012).
Though PD was first described in 1817, its pathogenesis is not fully defined yet. Disruption of neuronal homeostasis by various factors such as protein aggregation, mitochondrial dysfunction, inflammation and oxidative stress, are closely associated with PD (Dauer and Przedborski 2003).

2.1. α-syn aggregation

α-syn is apparent in central nervous system, which comprises approximately 1% of the total cytosolic proteins. Immunohistochemical analysis revealed that α-syn staining was observed in pre-synaptic terminals of the neurons (Kahle 2008). The role of α-syn in the pathogenesis of PD was characterized since the discovery of three familial forms of α-syn gene mutations. This includes mutation at 53\textsuperscript{rd} position in alanine to threonine substitution (A53T), 30\textsuperscript{th} position in alanine to proline substitution (A30P) and 46\textsuperscript{th} position in glutamate to lysine substitution (E46K) (Polymeropoulos et al. 1997; Kruger et al. 1998; Zarranz et al. 2004).

Several lines of evidences suggested the localization of α-syn in SNpc and ST brain regions and its role on membrane-associated processes at presynaptic level (George et al. 1995; Jenco et al. 1998; Chen et al. 1997; Abeliovich et al. 2000; Rajagopalan and Anderson 2001). Ueda et al. (1993) first described the association between α-syn and neurodegenerative disorder. Abnormal aggregation and accumulation of α-syn genes (A53T and A30P) led to the loss of dopaminergic neurons and the disease progression in PD (Kruger et al. 1998).

Common pathological features of surviving neurons are the presence of LBs and LNs in SNpc regions of PD (Kahle et al. 2000). LNs were found not only in SNpc regions but also in dorsal motor nucleus of the vagus, nucleus basalis of Meynert, and locus coeruleus (Forno 1996). It has been reported that α-syn is the major component of LBs and LNs (Conway et al. 1998). Thus, it is clear that α-syn aggregation is the common factor in the PD pathogenesis and its role in the disease process has been defined. Ubiquitin proteosome system (UPS) is the prime biochemical pathway, which is responsible for the degradation of normal and abnormal intracellular proteins, and evidences suggests that UPS system is impaired in PD (Gibb and Lees 1988; Ciechanover 2001). This evidently showed that UPS inactivity contributes to the development of PD forms.

The next important mechanism underlying α-syn aggregation is the involvement of oxidants that initiates the toxic intermediate oligimers, probably dopamine-α-syn adduct
(Hashimoto et al. 1999; Goldberg and Lansbury 2000). On the other hand, over-expression of α-syn stimulates iron dependent aggregation in in vitro studies (Osterrova-Golts et al. 2000). In addition, evidences support that over-activation of α-syn enhanced the vulnerability of neurons to dopamine induced cell death through the intracellular ROS generation (Rabinovic et al. 2000; Junn and Mouradian 2002; Zigmond et al. 2002). Xu et al. (2002) reported that accumulation of α-syn triggered apoptosis in cultured dopaminergic cells. The dopaminergic specificity of α-syn neurotoxicity is directly related to the dopamine and subsequently ROS production, which was evident from the inhibition of dopamine synthesis by TH inhibitor and prevented the α-syn associated apoptosis in cultured dopaminergic neurons (Xu et al. 2002). (Figure 2.2)

![Diagram](Modified from The FASEB Journal, 2004, vol. 18, pp. 620)

However, various studies imply the association of α-syn and PD through multiple and complex pathway, the clear conditions under which it remains toxic is ill-defined yet. A complete understanding on α-syn biology is essential for the development of novel therapeutic strategies in PD.
2.2. Mitochondrial dysfunction

Mitochondria is a highly dynamic organelle which contributes not only to the energy metabolism but also to the regulation of calcium homeostasis and programmed cell death. Since mitochondria plays a vital role in neuronal viability, its dysfunction leads to cell death, which points to the pathogenesis of PD. Mitochondrial dysfunction particularly in complex I links to PD pathogenesis. This involvement was first emerged from the accidental MPTP exposure to the drug abusers, which resulted in acute and irreversible Parkinsonism syndrome (Langston et al. 1983). Subsequent study on MPTP induced non-human primates and mice brain showed an abnormal dopaminergic neuronal loss in SNpc region (Dauer and Przedborski 2003). The link between the mitochondrial dysfunction and PD was further evidenced by administration of rotenone, a potent mitochondrial complex I inhibitor in rats (Betarbet et al. 2000).

Tieu et al. (2003) demonstrated that supply of chemicals / drugs directly to complex II, bypassing complex I blockade, enhancing oxidative phosphorylation and ameliorates dopaminergic degeneration in MPTP mice brain. In addition, virally mediated expression of yeast’s single-unitNADH-quinone oxidoreductase which is insensitive to complex I resulted in the attenuation of dopaminergic degeneration in rotenone induced PD (Marella et al. 2008). Also, methylene blue, an alternative electron carrier to cytochrome c attenuated mitochondrial dysfunction, motor deficits and dopaminergic cell death in rotenone induced rats, thus bypassing complex I blockade (Wen et al. 2011). This clearly states that mitochondrial impairment usually occurs in PD pathogenesis and the main consequences of this impairment might be the loss of ATP production and generation of ROS (Chan et al. 1991; Zhou et al. 2008).

Mitochondria play a key role in the regulation of programmed cell death as it contains various components in it. Abnormality in mitochondria activates caspase dependent or caspase independent pathways further leading to programmed cell death. It was reported that mitochondrial induced programmed cell death occured either by preventing B-cell lymphoma 2 (BCL2) families (BCL2 and BCL-xL) or by stimulating BCL2-associated X protein (BAX) expression (Vila and Przedborski 2003). In MPTP intoxicated mice, a time dependent activation of cytochrome C was observed followed by the activation of caspase 9, caspase 3 and apoptotic induced nigral cell death (Perier et al. 2005). Further, evidences support that dopaminergic cell death in MPTP mice was attenuated by targeting the molecules of mitochondrial pathway, such as caspase 9 or Apoptotic protease activating
factor 1 (APAF1) (Mochizuki et al. 2001; Viswanath et al. 2001) or by overexpressing BCL2 (Offen et al. 1998; Yang et al. 1998). (Figure 2.3)

Figure 2.3. Mitochondrial dependent apoptotic pathway in PD (Modified from Cold Spring Harbor Perspectives Medicine, 2012, vol. 4, pp. 14)

2.3. Neuroinflammation

The role of inflammation in the progression of PD has been well documented. Teismann and Schulz (2004) suggests that inflammatory mediators such as tumor necrosis factor α (TNFα), nitric oxide (NO) and interleukin 1 (IL-1) which are derived from microglia and astrocytes modulates the disease progression. Neuroinflammation once initiated will become self-sustainable which further triggers inflammatory pathways in SNpc region (Klegeris and McGeer 2007).

Initial evidence of neuroinflammation initiated from a post-mortem study, which demonstrated the presence of activated microglia in SNpc brain region of PD patient (McGeer et al. 1988). Subsequently, abundance of evidence supports the role of neuroinflammation and activated microglia in the pathology of PD (Banati et al. 1998; Gao and Hong 2008; Long-Smith et al. 2009; Hirsch and Hunot 2009; Imamura et al. 2003; McGeer and McGeer 2004; Orr et al. 2002). Activated microglia are largely found in SNpc
region of degenerating dopaminergic neurons in PD brains (Banati et al. 1998; Imamura et al. 2003; McGeer et al. 1988; Sawada et al. 2006). Various research groups also demonstrated the presence of activated microglia in rat brains lesioned with 6-hydroxydopamine (6-OHDA) (Crotty et al. 2008; Depino et al. 2003; He et al. 2001). ICAM-1, a key marker and trigger of inflammatory pathway was highly expressed in areas of neuronal loss in SNpc region of PD patients (Miklossy et al. 2006). Previous studies reported the increased expression of pro-inflammatory cytokines, IL-1β, TNF-α and IL-6 in PD patients (Boka et al. 1994; Dobbs et al. 1999; Mogi et al. 1994). In addition, enzymes associated with inflammation like inducible nitric oxide synthase (iNOS) and COX-2 were identified in post-mortem in PD brains (Hunot et al. 1997; Knott et al. 2000). (Figure 2.4)

![Figure 2.4. Inflammatory pathway in PD](Adapted from Annals of Neurology, 2003, vol. 53, pp. S55)

2.4. Oxidative stress
In both idiopathic and genetic PD, oxidative stress is the common mechanism in the cellular dysfunction. It is noted that increased oxidized levels of lipids (Bosco et al. 2006), proteins and DNA (Nakabeppu et al. 2007) and decreased levels of glutathione were observed in SNpc region of PD patients. The major source of oxidative stress was found to be produced during dopamine metabolism, mitochondrial dysfunction and neuroinflammation. (Figure 2.5)
Growing evidences have illustrated the role of oxidative stress in damaging the dopaminergic neurons (Rabinovic et al. 2000; Betarbet et al. 2002; Muftuoglu et al. 2004; Bender et al. 2006; Caudle et al. 2008). The most common cellular free radicals are hydroxyl radical, superoxide radical and nitric oxide. Compared to other organs, brain has some disadvantages in the detoxification of ROS

i. Cells of human brain consumes approximately 20% of the oxygen but it constitutes only 2% of the body (Clarke and Sokolo 1999), indicating the potential generation of a high quantity of ROS

ii. Iron content was reported to be high in some brain regions (Gerlach et al. 1994), which catalyses the formation of ROS

iii. High lipid content on brain targets to lipid peroxidation by ROS (Halliwell 1992)

iv. Low levels of anti-oxidants such as SOD, catalase, glutathione peroxidase (GPx) etc., in brain regions
Evidences showed that glutathione (GSH) plays a main role in defense mechanism against ROS. Injection of MPTP or 6-OHDA resulted in decreased brain glutathione content (Pileblad et al. 1989; Wullner et al. 1996). These results have been considered in the context of PD pathogenesis, where a lowered GSH content was found in SNpc brain region (Sofic et al. 1992). Thus it is clear that GSH, more abundant in astrocytes is essential in the detoxification of ROS as well as it may suppress the other molecular cascades in the progression of PD pathology.

2.5. Animal models of PD

For the past few decades, various animal models have been developed to elucidate the molecular events and their interrelations in PD pathogenesis and to study the neuroprotective effects of variety of drugs to ameliorate these alterations as well. Those include environmental, synthetic neurotoxins or genetic models.

2.5.1. Neurotoxic models

Neurotoxins like MPTP, 6-OHDA, rotenone and paraquat are most widely used to induced PD pathology (Figure 2.6).

![Figure 2.6. Neurotoxin models that induce PD pathology (Modified from The textbook: Mental and Behavioral Disorders and Diseases of the Nervous System, 2013, pp. 497)
2.5.1.1. MPTP
Though MPTP induced PD was accidentally discovered from drug abusers, today it represents a most frequently used PD animal model worldwide. It was repeatedly demonstrated that MPTP, a gold standard for toxic based animal models of PD among PD researchers replicates almost all the hallmarks of PD viz., oxidative stress, energy failure and neuroinflammation (Langston et al. 1983). Also, MPTP was shown to block neuromuscular junction through a curare-like action, by binding to the nicotinic acetylcholine receptors of the mouse diaphragm (Hsu et al. 1993). But this aspect of research is beyond the scope of the present experimental design.
MPTP, highly lipophilic molecule easily crosses blood brain barrier and enters astrocytes, where it gets converted to active metabolite – 1-methyl-4-phenylpyridinium (MPP⁺) by monoamine oxidase B (Cui et al. 2009). This active metabolite reaches dopaminergic neurons through dopamine transporter (DAT) and stored in vesicles (Javitch et al. 1985). Inside the neuron, MPP⁺ blocks the complex I activity, thereby depleting energy production and increased ROS generation. MPP⁺ in vesicles expels the stored dopamine contents into cytosol, which readily metabolizes and produce ROS finally depletion of dopamine content in neurons (Burke et al. 2008; Panneton et al. 2010). MPTP model usually performed in non-human primates and mice (Zigmond et al. 1989). MPTP has been administered in wide variety of dose regimens, but systemic administration resulted in reproducible results and the behavioral and neuroanatomical alterations in MPTP intoxication was found to be similar with that of human pathology (Langston et al. 1984; Przedborski et al. 2001). This model is useful in understanding molecular mechanism, and testing neuroprotective therapies. Currently, this model has become the standard-bearer for all toxin, based PD animal models.

2.5.1.2. 6-OHDA
6-OHDA is another commonly used animal model of PD (Schwarting and Huston 1996a; Schwarting and Huston 1996b; Schwarting and Huston 1997). Ungerstedt (1968) first demonstrated the use of this model in lesioning nigro-striatal pathway and this remains commonly used until today in both in vitro and in vivo. Rat, mice, dogs, cats are sensitive to 6-OHDA when injected intracerebrally, as it does not cross the blood brain barrier (Roeling et al. 1995; Valette et al. 1995; Annett et al. 1997; Ruffy and Leonard 1997). Even though 6-OHDA is similar to dopamine, the presence of hydroxyl group resulted in
the dopaminergic neuronal toxicity. Injection of 6-OHDA degrades approximately 60% of the tyrosine hydroxylase (TH) containing dopaminergic neurons in SNpc brain region (Blandini et al. 2008). Several studies evidenced that 6-OHDA injection depleted ST regions prior to the TH positive neurons in SNpc regions which replicates the PD in humans (Sauer and Oertel 1994; Przedborski et al. 1995). But the magnitude of lesion depends on the amount of toxin injection and the site of injection which further confirmed that this model does not mimic all the clinical features of PD. Dopamine depletion and SNpc degeneration was achieved in the injected site of the animal but the other brain regions was found to be unaffected. One of the main advantage in unilateral 6-OHDA model is that each animal can serve as its own control as there will be both lesioned and unlesioned hemisphere which is useful in behavioral analysis (Ungerstedt 1968).

2.5.1.3. Paraquat/Rotenone model

Paraquat and rotenone induced neurodegeneration was less widely used model since it does not mimic all the clinical features of PD. Though paraquat has structural similarities with MPP⁺, epidemiological reports suggest that pesticide increased the risk of developing PD, but only 95 cases of PD linked to its toxicity in humans (Berry et al. 2010). The importance of this model for PD researchers is that paraquat induced α-syn aggregation in every dopaminergic neurons and its ability to induce LB-like structures in SNpc (Manning-Bog et al. 2002). Chronic exposure to low doses of rotenone resulted in inhibition of the mitochondrial electron transport chain in the rat brain (Betarbet et al. 2000; Inden et al. 2011). Alam et al. (2004) showed behavioral and neurochemical deficits in rotenone injection, although mortality was very high. A study revealed that rotenone is not only specific to dopmainergic system but also produced deleterious effects on other neuronal population (Hoglinger et al. 2003). However, when rotenone was injected at lower doses chronically, it produced nigrostriatal lesions only in 50% of rats (Betarbet et al. 2000). Thus, it is not clear that these models showed any advantages over other toxic models, MPTP or 6-OHDA.

2.5.2. Genetic model

The main principle of the genetic model is to identify the molecular and biochemical pathways in disease progression. Genetic mutations are rare and characterized only 10% of all PD cases (Dauer and Przedborski 2003). The mutations in autosomal dominant gene -
α-syn and LRRK2 and autosomal recessive gene - PINK1/Parkin and DJ-1 are important to study potential therapeutic targets. Mutations in α-syn, which normally plays a role in synaptic vesicle, were the initial evidence for genetic link to PD. Mutations in A53T, A30P caused a dominantly inherited form of PD (Kruger et al. 1998). Studies on α-synuclein transgenic mice showed that A53T mutations in mice resulted in severe motor phenotype and finally led to paralysis and death (Giasson et al. 2002). In addition, mutations to α-syn in mice produced LBs inclusions (Masliah et al. 2000). Mutations in LRRK2 gene or knocking out LRRK2 gene showed no effect on dopaminergic neuron degeneration similar to α-syn (Wang et al. 2008). Therefore, LRRK2 mouse model is not preferably a suitable model since only minimal levels of neurodegeneration occurs (Li et al. 2009). Mutations in parkin, DJ1 and PINK1 did not show any nigrostriatal degeneration in rodent models or displayed any form of dopaminergic cell loss that resembles idiopathic or inherited PD. These models also failed to develop behavioral or pathological phenotype of PD (Moore and Dawson 2008). In general, this genetic mouse models were not able to produce the neuronal degeneration associated with PD and therefore these models might be defective and requires additional modulations or modifications (Peng et al. 2010).

2.6. Astrocytes and PD

Astrocytes are the major cell population, which constitutes about 50-60% in central nervous system (CNS). Their main role in the normal functioning of CNS is the regulations of blood flow, providing energy metabolites to neurons, participates in synaptic function by synchronizing neuronal firing patterns and maintenance of extracellular ions, fluids and transmitters (Volterra and Meldolesi 2005). Glial fibrillary acidic protein (GFAP), major intermediary filament component of astrocytes is involved in the astrocyte-neuronal interactions (Eng et al. 2000).

Astrocytes plays a major role in differentiation, survival, pharmacological properties and resistance to injury of dopaminergic neurons (Mena and de Yebenes 2008). High levels of intracellular adhesion molecule 1 (ICAM-1), an inflammatory mediator were observed in astrocytes of ST brain region, which makes this region more susceptible to inflammatory processes (Morga et al. 1998). During neuronal insult, astrocytes undergo various molecular and morphological changes. A key marker in astrogliosis is the upregulation of GFAP (Eddleston and Mucke 1993). Various aspects of astrogliosis play a vital role in the progression of PD. Though astrogliosis might be beneficial in maintaining extracellular
glutamate levels in ST after dopaminergic neuronal loss, its normal function is compromised during astrogliosis. Glutamate transporter, which is essential in astrocytes for the supply of glutathione precursor to neurons, is found to be reduced in chronic PD (Dervan et al. 2004). Alterations in astrocytic regulation of synaptic function and glutamate content links to the progressive nature of the pathophysiology associated with PD.

During stimulation of inflammatory cytokines in astrocytes, Ca$^{2+}$ independent $\text{iNOS}$ gets induced which results in the generation of NO (Bolanos et al. 1994). NO further induces the generation of ROS by damaging electron transport chain in mitochondria leading to cell death (Bolanos et al. 1994). On the other hand, increased NO depletes GSH content in astrocytes further leading to the loss of glutathione precursor supply to neurons and ROS over-production in chronic $\text{iNOS}$ induction (Sian et al. 1994; Heales et al. 2004). This decrease in GSH content precedes other hallmarks of PD pathogenesis. Gegg et al. (2003) reported a greater inhibition of mitochondrial function in neurons than in astrocytes exposed to NO.

Increased $\text{iNOS}$ expression in astrocytes might play a role in inducing GFAP expression, a major hallmark of astrogliosis. It has been noted that NO stimulated GFAP expression independent of the inflammatory mediators or the $\text{iNOS}$ stimulation in PD brain (Brahmachari et al. 2006). Brahmachari et al. (2006) also revealed that the expression of GFAP in astrocytes is increased through NO-GC-cGMP-PKG pathway, an important cascade in neurodegenerative conditions of PD.

### 2.7. RAS and PD

In addition to well defined pheripheral RAS, accumulating evidences showed the presence of RAS in central nervous system (Fischer-Ferraro et al. 1971; Stragier et al. 2008). In connection to this, various studies have focused central RAS to neurodegenerative diseases such as ischemia, PD, Alzheimer’s disease, depression, etc (Phillips and de Oliveira 2008). However, PD is the second most common neurodegenerative disorder, current therapies remains purely symptomatic (Dunnett and Bjorklund 1999; Johnston and Brotchie 2004). Until today, much effort has been made to identify the exact cause of the dopaminergic cell death in PD. Researchers suggested that oxidative stress, inflammation, mitochondrial dysfunction and excitotoxicity are the key players in the progression and pathogenesis of PD (Jenner and Olanow 1998; Anderson 2004; Block and Hong 2005). Numerous studies
implies that glial activation play a crucial role in neurotoxin induced animal models of PD. MPTP, a well known neurotoxin which triggers glial activation and nicotinamide adenine dinucleotide phosphate (NADPH) derived free radicals in both human and animal models of PD (Gao et al. 2002; Gao et al. 2003; Wu et al. 2003). Since angiotensin II is the pro-inflammatory compound that activates NADPH oxidase complex and free radical generation, regulation of brain RAS might contribute to slow down of the disease progression (Griendling et al. 2000; Griendling and Ushio-Fukai 2000).

2.7.1. Components of RAS
Presence of renin and angiotensin converting enzyme (ACE) has been demonstrated in various immunohistochemical techniques. ACE is localized in synaptosomal fraction of brain tissue with high concentrations in the lamina terminalis and the circumventricular organs, hypothalamus and some brain stem nuclei. Angiotensinogen is found in glial cells, its peptides were found in some neuronal population (Unger et al. 1988; Saavedra 1992). This suggested that angiotensin II is formed in extracellular brain regions and angiotensin I is present in less concentration. These data explains that angiotensinogen II is directly formed from angiotensinogen, rather than renin activity. Alternatively, non-ACE pathways were also demonstrated in brain areas (Saye et al. 1993; Phillips and Sumners 1998).

2.7.2. ACE inhibitors
ACE is widely distributed in brain including nigrostriatal pathway and in basal ganglia (Strittmatter et al. 1985; Skidgel and Erdos 1987; Chai et al. 1987; Chai et al. 1990). Several studies point towards the role of ACE in the pathogenesis of PD. Konings et al. (1994) demonstrated an increased ACE activity in cerebrospinal fluid of PD patients. Jenkins et al. (1997) studied the effect of perindopril, an ACE inhibitor, which revealed that chronic administration of perindopril improved the striatal dopamine content in rats. Perindopril treatment in six moderately severe PD patients resulted in the improvement of motor function as well (Reardon et al. 2000). In addition to the enzymatic cleavage of angiotensin I, ACE also metabolise bradykinin and in turn inflammation, a key factor in PD (Ehlers and Riordan, 1989). Lin et al. (2002) described an association between genetic polymorphism of ACE gene and PD in Taiwan. On the other hand, no correlation was observed between ACE gene and PD in Australian and Italian population (Mellick et al. 1999; Pascale et al. 2009).
Based on these arguments, researchers primarily focus on evaluating the neuroprotective and neurorestorative effects of ACE inhibitors in animal models of PD. Pre-treatment with perindopril decreased the loss of dopaminergic neurons in SNpc and the dopamine depletion in ST regions of MPTP intoxicated mice brain (Kurosaki et al. 2005). On the other hand, similar results were obtained in cardiopril administration against MPTP and 6-OHDA rodent model of PD (Lopez-Real et al. 2005; Munoz et al. 2006). This clearly suggests that these neuroprotective roles are not a unique property of perindopril, but probably complete ACE inhibitors characteristics.

Modulation of AT1R or AT2R by angiotensin II could be the possible reason for the restorative effect in animal models of PD. Brown et al. (1996) reported that acute administration of angiotensin II increased ST dopamine release via AT1R.

### 2.7.3. AT1R antagonists

It was reported that AT1Rs were present in SNpc and ST brain regions (Simonnet et al. 1981; Allen et al. 1998; Daubert et al. 1999). Several researchers also pointed towards the interaction between RAS and PD by modulating striatal dopamine content by AT1R (Mendelsohn et al. 1993; Brown et al. 1996; Ge and Barnes 1996). Activation of AT1R has been associated with the stimulation of NADPH oxidase complex, which in turn induce ROS generation. Administration of ZD7155, an AT1R antagonist reduces lipid peroxidation and protein oxidation in SNpc and ST regions of 6-OHDA intoxicated rat model of PD (Rey et al. 2007). Rey et al. (2007) also compared the effect of apocynin, an NADPH oxidase inhibitor against 6-OHDA induced dopaminergic neurodegeneration, which showed that the neuroprotective action of AT1R blocker has been mediated through NADPH oxidase complex. Studies also demonstrated that AT1R not only regulate the NADPH oxidase induced ROS, but also the activation of glial cells after 6-OHDA induction. This effect was not restricted to 6-OHDA model, it also showed neuroprotective effect in MPTP model as evidenced by losartan administration (Grammatopoulos et al. 2007).

Interestingly, AT1R blocker, in addition to suppressing NADPH oxidase activity, increased angiotensin II synthesis as well which in turn leading to the activation of AT2R (Steckelings et al. 2005; Grammatopoulos et al. 2005). Based on these observations, AT1R blocker might be more beneficial than ACE inhibitors in ameliorating the PD pathogenesis. In addition, AT1R blocker has more tendency to cross blood brain barrier and block
AT1Rs than ACE inhibitors (Gohlke et al. 2002), while leaving AT2R stimulation unaffected.

AT1R and AT2R exert opposing effects. One of the roles of AT2R is the protective action against over-activation of AT1R (Steckelings et al. 2005). Data suggested that AT2R expression was found to be increased in various pathological conditions including neurodegenerative diseases which favors the repair mechanism (Steckelings et al. 2005, Li et al. 2005). Consistent with this, it was reported that AT2R inhibits NADPH oxidase activity (Sohn et al. 2000; Chabrashvili et al. 2003).

2.7.4. AT2R agonists

AT2R is distributed in SNpc and ST brain regions and it plays a main role during brain development and tissue regeneration (Gendron et al. 2003; Reinecke et al. 2003). Studies on AT2R agonist implies that activation of AT2R increased cell proliferation, cell differentiation and tissue regeneration in different cell lines from neuronal origin (Cote et al. 1999; Laflamme et al. 1996; Meffert et al. 1996; Stroth et al. 1998; Li et al. 2005). In mesenteric precursor cells, angiotensin II is involved in the differentiation of precursor cells into dopaminergic neurons via AT2R activation. Thus researchers suggested that mixture of angiotensin II with different factors that induce cell survival and differentiation might lead to the large scale production of dopaminergic neurons for clinical transplantation in PD patients (Rodriguez-Pallares et al. 2004).

From the above findings, it is clear that blockade of AT1R may be more beneficial than ACE inhibitors or AT2R agonist in ameliorating the disease pathogenesis, as AT1R not only blocks NADPH oxidase activity but also stimulates AT2R action. Thus the present study was undertaken to study the role of AT1R blocker, TEL on neurotoxin induced dopaminergic neurodegeneration and glial activation in MPTP mice model.