Chapter 1

Review of Literature
1.1. Parkinson’s disease

PD is the second most common progressive neurodegenerative disorder after Alzheimer’s disease. The disease is mainly characterized by the resting tremor, rigidity, bradykinesia and postural instability (Yadav et al., 2012). The main anatomical feature of PD is the degeneration of dopaminergic neurons in the SNpc region of midbrain (Thomas and Beal, 2007; Srivastava et al., 2010). The loss of dopaminergic neurons results in the depletion of dopamine level in the striatum leading to loss of control over the movement (Yadav et al., 2012). The formation of intracytoplasmic inclusions commonly called as Lewy bodies, is the main pathological symptom of this deadly and baffling disease (Gibbs and Lees, 1988). The clinical symptoms of PD appear after the loss of 60-70% or more of the dopaminergic neurons (Yadav et al., 2012). The secondary and nonmotor symptoms of PD, such as, freezing, festination, shuffling gait, olfactory dysfunction, dementia, sleep disturbance, depression, anxiety etc., may appear at the later stage of the disease (Toulouse and Sullivan, 2008).

1.2. History

Swedenborg in 1740 wrote a book entitled “Oeconomia Regni Animalis” and described about the importance of the striatum. He stated “Royal road of the sensations of the body to the soul is through the corpora striata and all the determinations of the will is descended by that road” (Reid, 1990). James Parkinson, a British physician, for the first time described PD as triad of akinesia, rigidity and resting tremor in his monograph “An essay on the shaking palsy” in 1817 (Parkinson, 2002). The term “Parkinson’s disease” was given by Jean-Martin Charcot, a French neurologist, who is also called as the father of modern neurology (Goetz, 1986). Despite the fact that sporadic PD is known from several
years, the first case related to environmental origin of PD came in to the lime light in 1980s, when the PD like features were found to be present in the synthetic heroin (meperidine) addicts. Langston et al., identified that MPTP, a byproduct present in the synthetic heroin, as the main culprit of the appearance of PD like features in the addicts (Langston et al., 1983).

1.3. Epidemiology

The incidences of PD vary from population to population. It is established that PD affects populations of all age groups. However, adults above the age of 50 years are mainly affected from the disease. The number of cases in US was estimated at 340,000 in 2005 and is predicted to rise to 610,000 by 2030 (Dorsey et al., 2007). However, the low prevalence of PD is reported in Indians, except Parsis, as compared with the rest of the world (Singhal et al., 2003).

1.4. Etiology

PD can be contributed by several known and unknown risk factors. About 90% of the cases of PD are sporadic, with no clear etiology and 10% have a genetic origin. Several factors are involved in PD pathogenesis that includes aging, environmental and genetic factors. Family history, male gender and lifestyle factors are also found to be important contributory factors (Wooten et al., 2004; Golbe et al., 1990).

1.4.1. Age

PD is mainly known as an aging related disease, since aging is one of the main culprits (Yadav et al., 2012). The incidence and prevalence of PD increase with the age (Twelves et al., 2003; Simuni, 2007). Based on the time and cause of onset, PD is divided into three main categories: idiopathic PD (>40 years), young-onset PD (21–40 years) and juvenile
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PD (<20 years). The incidence of PD increases from 1-2% in the individuals of above the 60 years of age to 4-5% by the age of ~85 years (Aschner, 2000). Animal studies have also suggested that aging is associated with an increased activity of monoamine oxidase B (MAO-B), which catalyses the oxidative deamination of monoamines, an important enzyme involved in PD pathogenesis (Irwin et al., 1992).

1.4.2. Genetic factors

Onset of PD in young individuals below the age of 20 years has prompted the hypothesis that genetic factors play critical role in PD pathogenesis. The genetic polymorphisms in several genes have been experimentally studied and 16 PARK loci (PARK 1 to PARK 16) have been identified to be associated with PD till date (Lesage and Brice, 2009). Mutations in these genes have been found to cause Parkinsonian syndromes, which resemble sporadic or idiopathic PD (Fahn and Sulzer, 2004). In addition to PARK genes, pituitary homeobox 3 (Pitx3) and nuclear receptor related 1 protein (Nurr1) are also involved in the PD pathogenesis.

1.4.2.1. Dominantly inherited mutations

Mutation in PARK 1 and PARK 4 (α-synuclein), PARK 3, PARK 5 [ubiquitin c-terminal hydrolase-L1 (UCH-L1)], PARK 8 [Leucine-rich repeat kinase 2 (LRRK2)], PARK 9 (ATP13A2) and PARK 10 give rise to dominantly inherited form of PD (Yadav et al., 2012; Biskup et al., 2008). Among them, the roles of α-synuclein and LRRK2 have been widely studied in PD pathogenesis.

1.4.2.1.1. α-Synuclein

α-Synuclein, consists of 140 amino acids and plays significant role in learning, synaptic plasticity, vesicle dynamics and dopamine synthesis (Lotharius et al., 2002; Sidhu et al.,
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2004). Its N-terminus forms an amphipathic α-helical domain when associates with lipids. Three functional mutations 209G>A (Ala53Thr), 88G>C (Ala30Pro) and 188G>A (Glu46Lys) are reported in α-synuclein, which are associated with autosomal dominant Parkinsonism (Kruger et al., 1998; Zarranz et al., 2004). The mutations in α-synuclein gene reduce its affinity with lipids and promote self-assembling into oligomers, which participate in the formation of Lewy bodies.

1.4.2.1.2. LRRK2

LRRK2 is expressed in various regions of the brain, including the SNpc and dorsal striatum (Zimprich et al., 2004). LRRK2 contains 2527 amino acids and possesses five conserved C-terminal domains, which are involved in substrate binding, protein phosphorylation and protein-protein interactions. LRRK2 functions as cellular stress sensor (Meylan and Tschopp, 2005) and regulates cell-survival and inflammatory pathways. The most frequent mutation in LRRK2 at position 6099G>A (Gly2019Ser) is reported in the PD.

1.4.2.2. Recessively inherited mutations

Mutation in PARK 2 (Parkin), PARK 6 [PTEN-induced kinase 1 (PINK1)] and PARK 7 (DJ1) genes are found to be associated with recessive form of sporadic PD (Biskup et al., 2008; Valente et al., 2004; Kitada et al., 1998; Bonifati et al., 2003).

1.4.2.2.1. Parkin

Parkin contains 465 amino acids and possesses N-terminal ubiquitin-like domain and two RING-finger domains. Parkin functions as an E3-ubiquitin ligase and ubiquitinates the proteins by polyubiquitination through lysine 48 residue. Additionally, Parkin monoubiquitinated on the lysine 63 residue and degraded by the proteasome system
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(Kahle and Haass, 2004). The mutations in Parkin result in protein aggregation, however, its overexpression in various animal models has been reported to be neuroprotective in nature (Lo Bianco et al., 2004; Greene et al., 2005).

Figure 1.1. Possible effects of genetic defects in PD related genes (Adopted from Gao HM, Hong JS. Gene-environment interactions: key to unraveling the mystery of Parkinson's disease. Prog Neurobiol. 2011; 94: 1-19)
1.4.2.2. PINK1

PINK1 contains 581 amino acids protein (Valente et al., 2004) and protects neurons from stress-induced mitochondrial dysfunction and apoptosis (Deng et al., 2005). Mutation in PINK1 results in altered protein stability, localization and kinase activity (Petit et al. 2005; Beilina et al., 2005).

1.4.2.2.3. DJ1

DJ1 protein primarily exists as dimer and is localised in mitochondria. DJ1 plays critical role in D2 receptor-mediated dopaminergic neuronal transmission (Goldberg et al., 2005). Deletions and missense mutations at specific locations have been reported to induce oxidative stress (Tao and Tong, 2003; Zhang et al., 2005). Absence of DJ1 increases the sensitivity to oxidative stress, whereas its overexpression protects the neurons (Goldberg et al., 2005; Kim et al. 2005).

1.4.3. Environmental factors

The surroundings, which affect an individual directly or indirectly, is known as environment. The chemicals and compounds present in the environment that affect the PD pathogenesis are recognised as the environmental contributory factors. Environmental risk factors, such as heavy metals and pesticide have long been implicated in the etiology of PD (Lai et al., 2002). Various studies have demonstrated the positive correlation of PD with exposure to Mn, copper (Cu), mercury (Hg), lead (Pb), iron (Fe), aluminium (Al) and zinc (Zn) (Montgomery, 1995). Exposure to pesticides, well water drinking, farming and rural living are reported to increase the incidences of PD (Thiruchelvam et al., 2002). Environmental toxins and infectious agents (bacteria and viruses) provoke excessive production of ROS that may lead to neuronal cell damage by interacting with DNA (deoxyribonucleic acid), proteins and lipids (Cohen and Doherty, 1987). Pesticides
inhibits mitochondrial complex I and III of electron transport system and make nerve cells more vulnerable (Di Monte, 2003; Thiruchelvam et al., 2005).

1.4.4. Gender bias

The incidences of PD are reported to be higher in men than in women. A meta-analysis has shown that the male to female ratio of PD occurrence is 1.49 (Wooten et al., 2004). Possible explanation for the low prevalence in females could be the protective effect of estrogens in women (Wooten et al., 2004; Shulman, 2007). The role of estrogens in the protection of nigrostriatal dopaminergic neurons is also reported even in the animals (Dluzen et al., 1996). Other possible explanation of high prevalence in male could be higher exposure to toxins, frequent minor head trauma and the presence of recessive susceptibility genes on the X-chromosome in men (Wooten et al., 2004; Taylor et al., 2007).

1.4.5. Lifestyle related factors

Progression of PD is found to be associated with the intake of foods, such as proteins, fats, and carbohydrates. Several studies have assessed the risk of PD in relation to various food items. PD patient have the habit of lower preference for nuts, plums and salad oil and higher preference for spicy, almonds and plums foods as compared with healthy controls (Golbe et al., 1990; Golbe et al., 1988; Vieregge et al., 1992). Intake of coffee or caffeinated products exerts neuroprotective effect against PD and acts as an adenosine receptor antagonist (Ho, 2002).

1.5. Animal models of PD

Animal models offer a number of advantages for studying human neurodegenerative diseases. Epidemiological evidences have shown a strong association between pesticide
exposure and increasing incidences of PD. Such findings have prompted investigators to develop pesticides-induced animal models to understand the molecular and biochemical events leading to disease and also to develop therapeutic strategies to encounter PD (McCormack et al., 2002; Patel et al., 2006; Thiruchelvam et al., 2002). Similarly, many genes, which regulate PD pathogenesis led to the development of genetic models.

1.5.1. Lower animals and plants

Lower animal or plant models, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Caenorhabditis elegans* (*C. elegans*), have been used to identify the genes and the drugs that could be therapeutically relevant to PD (Bilen and Bonini, 2005; Caldwell and Caldwell, 2008; Gitler, 2008).

*Drosophila* is considered as one of the best genetic models for PD. Although, *Drosophila* does not possess clear α-synuclein homologue and is therefore, overexpression of wild-type or mutant α-synuclein reproduces many critical features of PD, such as Lewy body-like aggregate formation, degeneration of dopaminergic neurons and impairment of locomotor activity (Feany and Bender, 2000; Singleton et al., 2003). Loss-of-function mutations or dopaminergic neuron-specific inactivation of the respective *Drosophila* homologues of PINK1 (Wang et al., 2006), Parkin (Whitworth et al., 2005), DJ1 (Faust et al., 2009), LRRK2 (Lee et al., 2007a) and high temperature requirement protein A2 (HtrA2) (Whitworth et al., 2008) could lead to Parkinsonian features (Hirth, 2010).

Among various cellular and transgenic models of PD, *C. elegans* is considered as an ideal model owing to its short life span, well-defined nervous system and transparent body. It has many advantages over rodents, primates and other lower animal models since its neuronal circuitry is fully mapped (Bargmann, 1998). It has only 8 dopaminergic neurons, which could be easily visualized by immunostaining with a fluorescent protein. Six
ortholog of *C. elegans*, such as PARK 2, PARK 5, PARK 6, PARK 7, PARK 8 and PARK 9 have been reported. Since, there is no ortholog for α-synuclein in *C. elegans*, therefore, wild type or mutant type could be overexpressed without any fear of its endogenous expression. α-Synuclein mutants (A30P and A53T) and its multiplication have been linked to increased α-synuclein aggregation and dopaminergic neuronal death (Mezey et al., 1998; Conway et al., 2000; Singleton et al., 2003; Ross et al., 2008). The overexpression of wild-type or mutant α-synuclein under the control of dopamine transporter (DAT)-1 promoter results in the loss of dopaminergic neurons. α-Synuclein expression may lead to a significant reduction in the motor movement of *C. elegans* (Lasko et al., 2003). Many α-synuclein mutants of *C. elegans* have been made with various promoters. Overexpression of either wild-type or G2019S LRRK2 under the control of pan-neuronal promoter (snb-1 promoter) enhances dopaminergic neurodegeneration accompanied by reduction of dopamine level (Saha et al., 2009). It is also considered as an experimental model to examine various environmental toxins, including 6-OHDA, MPTP, Mn, PQ and rotenone, which could induce the cardinal features of PD (Nass et. al., 2002; Braungart et al., 2004; Settivari et al., 2009; Samann et al., 2009; Ved et al., 2005). Despite many advantages, it has several disadvantages, its anatomy does not resemble with humans, therefore, extrapolation of the data obtained from this model to human is theoretically not feasible. Another limitation of this model is the presence of only 302 neurons against billions of neurons in the human brain (Bargmann, 1998).

### 1.5.2. Primates

Nonhuman primate models of PD are the key models to unravel PD pathophysiology and evaluate therapeutic strategies for the disease. The motor and cognitive skills and neuroanatomical complexity of primates closely resemble with humans, therefore, primates provide more detailed knowledge of PD pathogenesis, including clinical
outcomes (Capitanio and Emborg, 2007). Like humans, loss of striatal dopamine, decreased number of tyrosine hydroxylase (TH)-positive cells and DAT are reported in rhesus monkey (*Macaca mulatta*) (Collier et al., 2007; Emborg et al., 1998). Kirik and Eslamboli developed a genetic model by introducing the α-synuclein gene in the brain of adult common marmoset monkeys (*Callithrix jacchus*) to assess the role of α-synuclein in the nigral cell death (Kirik et al., 2003; Eslamboli et al., 2007). 6-OHDA and MPTP are known to induce PD like features in monkey (Eslamboli et al., 2003; Stephenson et al., 2005). Primate models are not able to decipher the genetically induced pathological symptoms of PD (Fleming and Chesselet, 2006).

### 1.5.3. Rodent models of PD

MPTP- and 6-OHDA-induced the PD phenotype in primate model but it is easy to find out the mechanism of PD pathogenesis in rodent models. Several pesticide-induced rodent models, such as MPTP, 6-OHDA, rotenone, amphetamine and MB and PQ are discussed in this section.

#### 1.5.3.1. MPTP

MPTP has widely been used to develop PD like symptoms in the rodents to understand the pathophysiology of PD and efficacy of anti-PD drugs. The development of this model is based on the historical observations. During 1980s, the synthetic heroin addicts in North California were found to suffer from phenotypic symptoms that resemble PD (Miller et al., 2009). Langston and his co-workers identified MPTP, a contaminant by product present in the synthetic heroin (Langston et al., 1983).

MPTP, a lipophilic molecule, readily crosses the BBB (Mayo et al., 2005). MPTP gets converted to 1-methyl-4-phenyl-2, 3-dihydropyridium in cells like astrocytes and serotonergic neurons by MAO-B, which in turn oxidizes to 1-methyl-4-phenylpyridinium
(MPP\(^+\)) ion, subsequently it is released into the extracellular space (Nicklas et al., 1985; Przedborski and Vila, 2003) and enters the dopaminergic neurons through DAT (Javitch and Snyder 1984). MPP\(^+\) inhibits the sequestration of dopamine by binding to the vesicular monoamine transporter (VMAT 2) (Del Zompo et al., 1993). MPP\(^+\) also accumulates within the mitochondria and inhibits the complex I (Nicklas et al., 1985) leading to generation of ROS, which are the major factors involved in the degeneration of dopaminergic neurons.

MPTP effectively elicits most of the cardinal features of sporadic PD in the non human primates and experimental rodents (Mayo et al., 2005; Langston et al., 1999). Although MPTP does not induce the formation of non-fibrillar Lewy bodies in the nigrostriatal tissues and many phenotypic symptoms in the rodents, such as resting tremor, it is known to degenerate the dopaminergic neurons by inducing oxidative stress. MPTP alters nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in addition to the activation of microglial cells and neuroinflammation (Gao et al., 2003). It induces apoptosis via the activation of caspases and augments DNA fragmentation (Kotake and Ohta, 2003; Schober, 2004).

1.5.3.2. 6-OHDA

6-OHDA is possibly the first chemical reported to degenerate the dopaminergic neurons of the nigrostriatal pathway in the experimental animals (Bove et al., 2005). 6-OHDA is not able to cross the BBB and enters the brain through direct stereotaxic injection to the nigrostriatal system. 6-OHDA is structurally similar to dopamine, therefore uses DAT to enter in the dopaminergic neurons. 6-OHDA produces free radicals either through auto-oxidation or oxidation mediated by monoamine oxidase-A (Padiglia et al., 1997). ROS production results in a rapid depletion of cellular antioxidant enzymes, leading to
abnormalities in the cell structure and metabolism and eventually results in neuronal damage.

6-OHDA accumulates in the neurons and causes degeneration of the cell bodies of the dopaminergic neurons in the SNpc and their fibres in the striatum (Bove et al., 2005). Additionally, 6-OHDA induces microglial activation in the nigrostriatal region (Croisier et al., 2005). It is known to produce several symptomatic features in the rodents mimicking sporadic PD, owing to its ability to induce free radical generation, inhibit mitochondrial electron transport chain complexes I and IV and to induce apoptosis (Bove et al., 2005; Glinka et al., 1997; Schober, 2004; Uversky, 2004). While, 6-OHDA cannot readily cross the BBB owing to its hydrophilic nature and it is not an environmental chemical to which humans are exposed in their day-to-day life, it is one of the most widely used neurotoxins for understanding PD pathogenesis and assessing the treatment outcomes.

1.5.3.3. Rotenone

Rotenone belongs to rotenoids, a family of natural cytotoxic compounds, extracted from various parts of *Leguminosa* plants and is widely used around the world as pesticide. As, rotenone is a lipophilic in nature, it readily crosses the BBB and enters the brain (Dauer and Przedborski, 2003). Rotenone crosses all cellular membranes and accumulates in the subcellular organelles, such as mitochondria. Rotenone inhibits the mitochondrial electron complex I and induces free radical generation, which could lead to microglial activation and apoptosis (Uversky, 2004; Gao et al., 2002).

Rotenone leads to the dopaminergic neurodegeneration and reduces the number of TH-positive neurons of the nigrostriatal pathway. Although degeneration caused by rotenone is non-specific in nature, it is the only neurotoxin, which exhibits well defined Lewy body formation and aggregation of α-synuclein along with depletion of glutathione, disruption
of axonal transport and onset of several critical histological, biochemical and pathological hallmarks of sporadic PD (Yadav et al., 2012; Bove et al., 2005; Lin et al., 2008; Uversky, 2004). Since uptake of rotenone does not depend on the DAT to exert neurotoxicity, it could be considered as an ideal model to assess the efficacy of neuroprotective aspects (Tapias et al. 2010).

1.5.3.4. Amphetamine

Methamphetamine-induced striatal dopamine depletion is considered as one of the important models to mimic some of the PD pathology (Gerlach and Reiderer, 1996). Chronic or intermittent methamphetamine/amphetamine induces temporary or permanent disturbance in the dopaminergic system leading to PD like symptoms (Virmani et al., 2002). Moreover, methamphetamine increases protein-1 and cyclic adenosine monophosphate response element binding protein expressions by activating the respective transcription factors and directly acting on the mesencephalic cell nuclei (Asanuma et al., 2000). Amphetamine leads to an increase in the level of α-synuclein and decrease in the phosphorylated TH and mitochondrial complex I proteins (Klongpanichapak et al., 2008).

1.5.3.5. MB and PQ

PQ, an herbicide, possesses structural similarity with MPP⁺, the primary metabolite of MPTP and induces the nigrostriatal dopaminergic neurodegeneration almost in the same fashion as MPTP (Uversky, 2004). Unlike MPTP, PQ is a polar molecule and crosses the BBB through neutral amino acid transporter (Shimizu et al., 2001). PQ undergoes one electron reduction and also accumulates within the cells, which results in oxidative or nitrosative stress (Berry et al., 2010). Because PQ is used widely in agriculture, concern has been raised that its exposure could increase the risk of developing PD in humans.
MB, a fungicide, induces the nigrostriatal dopaminergic neurodegeneration along with PQ much more potentially than PQ alone (Thiruchelvam et al., 2000a). PQ and MB inhibits the mitochondrial complex I and III, respectively (Patel et al., 2006). Both of them when administered together lead to free radical generation, inhibit proteasomal function and energy metabolism more potentially than that of alone (Thiruchelvam et al., 2000a). These pesticides generate superoxide, hydroxyl and fatty acyl radicals leading to DNA fragmentation and apoptosis (Patel et al., 2006; Patel et al., 2007; Patel et al., 2008; Peng et al., 2005; Shimizu et al., 2003; Thiruchelvam et al., 2000a). Independently, MB and PQ induce apoptosis through the activation of Bak (BCL2-antagonist/killer) pathway, however, in combination Bax (BCL2-associated X protein) pathway gets activated (Fei and Ethell, 2008).

Dysfunction of proteasome system and increase in α-synuclein aggregation are quite common in PD and the same are reflected in PQ-induced Parkinsonism in the experimental animal (Yang and Tiffany-Castiglioni, 2007; Manning-Bog et al., 2002). PQ-induced toxicity and proteasome dysfunction are potentiated by DJ1 deficiency/knock out (Yang et al., 2007). While mutation in LRRK2 causes familial PD, its overexpression has been shown to encounter PQ-induced toxicity (Saha et al., 2009).

PQ-induced neuronal cell death is accomplished by the intrinsic mitochondrial pathway, which is activated by a wide variety of other cytotoxic stimuli or environmental stressors (Franco et al., 2010). The PQ exposure activates Bax or Bak and increase outer membrane permeability of mitochondria and allows release of pro-apoptotic factors (Ethell and Fei, 2009). Oxidative stress caused by PQ leads to misfolded /unfolded protein accumulation leading to endoplasmic reticulum (ER) stress, which stimulate specific caspase dependent and independent apoptotic pathways (Chinta et al., 2008). Furthermore, PQ induced ER
stress leads to activation of caspase 3 and caspase 7 and cleavage of PARP-1 [Poly (ADP-ribose) polymerase 1] (Peng et al., 2005).

PQ induces microglial activation and inhibits NADPH oxidase activity (Croisier et al., 2005; Miller et al., 2009). Activated microglia secretes proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), which play critical roles in neuronal death (Tansey and Goldberg, 2010). It is reflected from the studies which have shown that knockdown of IFN-γ expression protects against PQ induced toxicity and inhibits the time dependent change in nitric oxide synthase (NOS), cyclooxygenase 2 (Cox2), c-jun N-Terminal Kinase (JNK), IL-1β and p38 expressions.

1.5.3.6. Metal-induced PD model

Transition metals are required for the synthesis of DNA, RNA (ribonucleic acid) and proteins and act as cofactors of numerous enzymes. Deficiency of metals can lead to disturbances in the central nervous system and their accumulation in the brain in excess could be cytotoxic, which increases free radical production and thereby oxidative stress (Sayre et al., 1999; Sayre et al., 2000). Epidemiological and experimental evidences have largely depicted the role of heavy metals in PD. Various studies have implicated a positive correlation of PD with exposure to heavy metals, such as Mn, Cu, Hg, Pb, Fe, Al and Zn (Patel et al., 2005). Co-exposure of metals (Pb, Cu and Fe) induces pronounced Parkinsonian features as compared to any of these metals alone (Gorell et al., 1995; Gorell et al., 1997).

Fe is the most abundant metal reported to present in the globus pallidus putamen and SNpc of the basal ganglia (Dexter et al., 1993; Youdim and Riederer, 2004). Changes in Fe content in the brain have been found to be associated with PD (Dexter et al., 1987). Fe-
mediates cellular toxicity occurs by the induction of free radical generation and thereby oxidative stress. Fe enhances the PQ-induced dopaminergic neurodegeneration through the activation of microglia and NADPH oxidase (Peng et al., 2009).

Although, Zn is essential for the maintenance of normal physiology, its excessive accumulation may lead to induce PD pathogenesis (Takeda, 2000; Dexter et al., 1991). Systemic Zn exposure is known to cause dopaminergic neurodegeneration in the adult rats (Kumar et al., 2010). Zn also induces alterations in the cytosolic and mitochondrial SOD (Singh et al., 2011).

1.5.3.7. Other toxicant and pesticide model

Several toxicant and pesticide, other than described in the previous sections, also induces the PD-like symptoms. Trichloroethylene and Annonacin, complex I inhibitors, are reported to cause nigrostriatal dopaminergic neurodegeneration and induce PD-like features (Gash et al., 2008; Lannuzel et al., 2003). Several bacterial neurotoxins, such as β-Methylamino-L-alanine and tetrahydroisoquinoline could also elicit PD phenotype (Holtcamp, 2012; Storch et al., 2002; Ohta et al., 1990).

Cypermethrin, a commonly used pesticide, degenerates the dopaminergic neurons in the SNpc region and depletes the dopamine level in the striatum region (Singh et al., 2010). It modulates the expressions and catalytic activities of the toxicant responsive genes, microglial activation and the expression of apoptotic and inflammatory proteins in the nigrostriatal region of the brain (Tiwari et al., 2010; Tiwari et al., 2012; Singh et al., 2011).
1.5.4. Genetic model of PD

The genetic models have been made in rodents, Drosophila and *C. elegans* to study the mechanism of PD pathogenesis and to develop therapeutic strategy to encounter the disease (Dawson et al., 2010). Different animal models have been made by the alteration in the PARK genes whether it is null mutation or addition of an extra gene copy or point mutation (Fleming et al., 2005; Melrose et al., 2006). The loss-of-function mutation in recessively inherited genes (Parkin, DJ1 and PINK1) and gain-of-function mutations in dominantly inherited genes (α-synuclein and LRRK2), are mainly involved in the PD pathogenesis (Dawson et al., 2010).

Several α-synuclein modified mouse strains have been produced by adding extra copy of α-synuclein or by mutation at A30P or A53T. In this model, PD key symptoms were not detected, but the presence of α-synuclein depositions can be correlated with neuronal damage (Richfield et al., 2002). Mouse models with mutant or wild-type LRRK2 overexpression or null mutation have not so much established.

Genetic model of PINK1, DJ1 and Parkin have been studied in the number of investigation and reported to be involved in the mitochondrial function. Mutation in these genes affects the function of mitochondria, which is essential for the survival of dopaminergic neurons. Double-knockout mouse model of Pitx3 or Engrailed (En) has been developed, which is critical for the development and survival of dopamine neurons (Nunes et al., 2003; Sgado et al., 2006). The genetic models related to PD-linked genes so far did not produce the key symptoms of the disease i.e. loss of dopaminergic neurons, however, reduced dopamine level in the striatum was detected (Fleming et al., 2005).
1.5.5. Fusion model of PD

Genetic models do not show the clear damage to the dopaminergic neurons and only show altered dopaminergic neurotransmission and other risk factors. Toxin models exhibited degeneration of the dopaminergic neurons in the SNpc but mostly do not exhibit all cardinal feature of PD, such as Lewy body formation and slow and progressive degeneration. Therefore, to develop better models for PD, investigators attempted combinatorial approach. Combinational/fusion model systems exhibit the gene-environment interactions and mediate chronic and progressive neurodegeneration mimic by sporadic PD.

Several fusion models have been made by the combination of neurotoxins and transgenic animals (transgenic for PARK 1, PARK 2 and PARK 6). Fusion model offer extensive mitochondrial damage, axonal degeneration and the formation of neuritic and cytoplasmic perinuclear inclusions in α-synuclein-MPTP fusion model system as compared with toxin model alone (Song et al., 2004).

Parkin deficient mouse did not show the neuropathological features of PD phenotype (Goldberg et al., 2003; Itier et al., 2003; Von Coelln et al., 2004; Perez and Palmiter, 2005), however, it can induce mitochondrial abnormalities and reduces expression of proteins involved in response to oxidative injury. Combinatorial approach with toxin may increase the effect of neurodegeneration produced by neurotoxins. Despite possibility, there are no reports of Parkin deficient mice that have been exposed to MPTP, PQ rotenone or LPS. α-Synuclein overexpression increases the vulnerability of nigral neurons to the toxic effects of PQ (Manning-Bog et al., 2002) but not in case of toxic effects of rotenone (Neito et al., 2006). DJ1 deficient mice would be more vulnerable to oxidative
injury induced by MPTP and amphetamine (Kim et al., 2005) but no alterations were observed in mice exposed to PQ (Goldberg et al., 2005).

### 1.6. Possible mechanisms underlying PD

The clear mechanisms of PD pathogenesis are not yet known. However, oxidative stress, protein aggregation, neuroinflammation and apoptosis could play important roles in the onset of PD pathogenesis (Yacoubian and Standaert, 2009).

#### 1.6.1. Oxidative stress

Brain is the central regulatory unit of the body and needs much more amount of oxygen to generate more energy as compare to other organs. During process, large amount of free radicals generate, which causes damage to DNA, RNA, proteins and lipids. Oxidative stress induces the expression of phase I and phase II enzymes, such as glutathione-S-transferase (GST), different isoform of cytochrome P-450 (CYPs), which could be involved in the PD pathogenesis. Auto-oxidation of dopamine and its metabolites promotes oxidative stress and ROS generation (Hastings et al., 1996). Increased Fe content in the SNpc promotes free radical production in the presence of neuromelanin (Dexter et al., 1989). Several genes, which are linked to familial forms of PD, such as PINK1 and DJ1, appear to be involved in protection against oxidative stress (Clark et al., 2006; Kim et al., 2005).

Not only MB and PQ but all the toxins suspected to induce PD in humans or induce PD phenotype in animals selectively target dopaminergic neurons of the substantia nigra. While the preferential selection of dopaminergic neurons in the substantia nigra by the Parkinsonian toxins are not yet completely known, several theories to the unique susceptibility of dopaminergic neurons in the substantia nigra have been proposed. The vulnerability of the substantia nigra is related to the higher tissue neuromelanin-associated
redox iron level, which is known to enhance the generation of ROS in the cells leading to neurodegeneration (Berg et al., 2002; Lotfipour et al., 2012; Hirch, 1992). Dopaminergic neurons contain neuromelanin, an auto-oxidation product of catecholamine. Similarly, high level of copper-zinc superoxide dismutase is found to be present in the substantia nigra, also suggesting the excessive production of reactive oxygen species in this tissue. The level of iron, which exacerbates the production of free radicals, is also found to be high in the substantia nigra. Moreover, dopaminergic neurons of the substantia nigra show abnormality in the mitochondrial complex I, causing aggregation of alpha-synuclein, which results in an abnormal protein handling system leading to neuronal death (Dawson and Dawson, 2003). Furthermore, dopaminergic neurons of the substantia nigra contain less calbindin as compared with other dopaminergic or non-dopaminergic neurons (Liang et al., 1996). Calbindin is involved in the calcium ion transport within the cells and excess calcium in the cells is highly toxic, which leads to neuronal cell death. The calbindin theory also explains the high cytotoxicity in the substantia nigra as compared with other tissues of the brain, including dopaminergic neurons located in the ventral tegmental area.

1.6.2. Misfolding and aggregation of proteins

Aggregation and misfolding of proteins have been emerged as the important mechanisms in PD pathogenesis. Mutations in α-synuclein (point mutation, gene duplication) promote the formation of protein aggregates, the Lewy bodies and Lewy neuritis, which are the main feature of sporadic PD (Irizarry et al., 1998; Spillantini et al., 1997). Lewy bodies and Lewy neurites could be responsible for apoptosis and neuroinflammation. The mutation in Parkin and UCH-L1 disturbs the proteasome function and promotes aggregation of damage or mutated or unwanted proteins within the cell, which could be possible reason for the initiation of apoptosis (McNaught et al., 2002; Chung et al., 2001).
1.6.3. Microglial activation and inflammation

Role of neuroinflammation is reported in PD pathogenesis as an increased expression of pro-inflammatory cytokines, such as IL-1β, interleukin-6 and TNF-α is reported (Mogi et al., 1994a; Mogi et al., 1994b). Activation of microglia has been demonstrated in the SNpc and striatum from post-mortem PD brains and in animal models (McGeer et al., 2003; Orr et al., 2005). Although, the exact mechanism of microglial activation is not known, cytokines and α-synuclein could aggregation promote microglial activation (McGeer and McGeer, 2007).

1.6.4. Apoptosis

Cell death occurs either by apoptosis or by autophagy and triggered by oxidative stress, protein aggregation and inflammation (Anglade et al., 1997; Hirsch et al., 1999). Endocytic, mitochondria mediated and exocytic apoptotic mechanisms are involved in PD pathogenesis. Mitochondria-mediated apoptosis plays critical roles in number of pesticide induced animal models. In the exocytic pathway, pro-inflammatory cytokines induce the apoptosis pathway.

1.7. Possible treatment of PD

Despite many drugs exhibit the partial protective effect in animal models of PD, there is no drug available till date which can cure PD. Drugs available in the market are the supplements of dopamine, dopamine receptor agonist, adenosine receptor antagonist, MAO-B inhibitors and catechol-O-methyl transferase (COMT) inhibitors, several anti-inflammatory and neurotrophic agents.
1.7.1. Levodopa (L-DOPA)

L-DOPA is one of the oldest and most effective therapies used for the symptomatic relief of PD (Murer et al., 1998; Datla et al., 2001). Although, it is one of the most widely used drug, dyskinesia has been reported after prolonged treatment because of dopamine catabolism, which produces free radicals and potentiates neurodegeneration (Fahn, 1996; Barzilai et al., 2001). The hypotensive effect of L-DOPA also has been reported in PD patient (Irwin et al., 1992b). L-DOPA is given in combination with carbidopa, as L-DOPA alone could be converted to dopamine by DOPA carboxylase and dopamine is not able to cross the BBB.

1.7.2. Dopamine receptor agonists

Dopamine itself is free radical generator, therefore, dopamine receptor agonists are used to suppress dopamine release by acting at D2 autoreceptors present on terminals of dopaminergic neurons. Dopamine receptor agonists have been extensively used to reduce dopaminergic cell death (Carvey et al., 1997; Kitamura et al., 1998; Ogawa et al., 1994). Pramipexole, a dopamine receptor agonist, acts as the direct antioxidant owing to the presence of hydroxylated benzyl ring structure (Ogawa et al., 1994).

1.7.3. MAO-B and COMT inhibitors

MAO-B metabolises dopamine and its inhibition can reduce dopamine oxidation. Selegiline, a MAO-B inhibitor, reduces dopamine oxidation and also acts as the antioxidant in clinical trials (Tetrud and Langston, 1989). Rasagiline, MAO-B inhibitor, is more potent than selegiline and its metabolites offer potential antioxidant properties (Youdim et al., 2005).
COMT inhibitors such as entacapone and tolcapone block peripheral conversion of L-DOPA to 3-O-methyl DOPA and increase both the plasma half life of L-DOPA and its availability in CNS. Entacapone and tolcapone also provide symptomatic relief from motor fluctuations in PD (Gershanik et al., 2003; Schrag, 2005).

1.7.4. Antioxidants and neurotrophic factors

Antioxidants reduce the oxidative stress and free radical generation. Several groups of antioxidants are reported that offer relief from the symptomatic feature of PD. Nicotine, caffeine, vitamin C and resveratrol have been used as potential antioxidants in the various rodents models (Singh et al., 2008; Srivastava et al., 2012). Glial cell-derived neurotrophic factor (GDNF), a potent neurotrophic factor, supports the survival of dopaminergic nigral neurons and is found to be neuroprotective in animal models for PD (Kordower et al., 2000; Lin et al., 1993).

1.8. Melatonin

Melatonin and its metabolites offer anti-inflammatory, anti-apoptotic, anti-oxidative and free-radical scavenging properties and protect against mitochondrial dysfunction (Esposito and Cuzzocrea, 2010; Galano et al., 2011; Leon et al., 2004; Mayo et al., 2005), thereby regulating multiple biological processes in the body of an organism. It is known to control the transcription, translation and catalytic activities of the preventive antioxidants, including GPx, SOD and catalase under physiological and stress conditions (Rodriguez et al., 2004). Melatonin reduces the expression of adhesion molecules and pro-inflammatory cytokines and regulates the expression of xenobiotic metabolizing enzymes and serum inflammatory indices (Esposito and Cuzzocrea, 2010). Furthermore, melatonin increases the activity of the mitochondrial complex I and complex IV, preserves homeostasis, improves respiration, enhances glutathione level, increases ATP synthesis and decreases
the harmful reduction in the mitochondrial membrane potential, which triggers mitochondrial transition pore opening and the apoptotic cascade (Leon et al., 2005; Srinivasan et al., 2005).

Melatonin offers neuroprotection in a number of neurodegenerative diseases, including PD (Mayo et al., 2005). Since melatonin is a powerful free-radical scavenger, naturally occurring antioxidant defence stimulator, potent anti-apoptotic agent and a modulator of xenobiotic metabolizing enzymes, therefore, it may be considered to ameliorate the symptomatic features of PD (Galano et al., 2011; Leon et al., 2004; Rodriguez et al., 2004). Melatonin is an ideal neuroprotective agent as it can easily cross the BBB and enter the subcellular compartments, lacks toxicity as compared with many other neuroprotective agents and possesses effective combating efficacy against free radical-induced neuronal injury (Gupta et al., 2003). The presence of a considerable amount of melatonin in the mitochondria and growing evidences for mitochondrial dysfunction and oxidative stress in the dopaminergic neurons raise the possibility of functional implication of melatonin in the mitochondrial and non-mitochondrial functions associated with PD (Escames et al., 2010). This is further supported by the fact that melatonin effortlessly enters the brain and cerebrospinal fluid owing to its smaller size and amphiphilic nature (Gupta et al. 2003).

Melatonin is widely accepted as an alternative approach to ameliorate the symptomatic features of PD in experimental animals (Borah and Mohanakumar, 2009; Klongpanichapak et al., 2008; Reiter et al., 2008; Tapias et al., 2009).

1.8.1. Source

Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine, is a highly conserved anti-oxidant molecule secreted from the pineal gland, gastrointestinal tract, ovaries, testis, bone
marrow and eye lenses (Esposito and Cuzzocrea, 2010). Melatonin is found in many plants including tomatoes, grape skins and walnuts.

![Chemical structure of melatonin](http://chemistry.about.com)

**Figure 1.2.** Chemical structure of melatonin (http://chemistry.about.com)

1.8.2. **Melatonin and toxin induced-animal model of PD**

1.8.2.1. **Melatonin and 6-OHDA**

Melatonin effectively prevents apoptosis and protects against cell death caused by both low and high doses of 6-OHDA (Mayo et al., 1999). Melatonin restores 6-OHDA-induced loss of TH-positive cells, i.e., the dopaminergic neurons in the SNpc, absence of terminals in the dorsolateral striatum ipsilaterally and behavioural deficits (Kim et al., 1998). Melatonin prevents lipid peroxidation and significantly recovers the striatal dopaminergic function by restoring TH activity and dopamine content in 6-OHDA induced PD in rats (Joo et al., 1998).

1.8.2.2. **Melatonin and MPTP**

Melatonin also ameliorates the progressive impairment of the mitochondrial function, prophylactically reduces the oxidative damage and lipid peroxidation in MPTP-induced PD (Absi et al., 2000; Jin et al., 1998; Reiter, 1998) and protects TH-positive nerve
terminals (Acuna-Castroviejo et al., 1997; Capitelli et al., 2008). Since melatonin easily enters the brain, therefore, it inhibits the damage elicited by the chronic administration of MPTP, as measured by the nigral cell count, TH protein level and other ultra-structural features related to PD pathology (Antolín et al., 2002).

1.8.2.3 Melatonin and rotenone

Melatonin prevents the nigrostriatal neurodegeneration and α-synuclein aggregation (Lin et al. 2008) induced by rotenone. Melatonin scavenges rotenone-induced hydroxyl radicals and restores the decreased glutathione level and changes in the catalytic activities of SOD and catalase in the SNpc (Saravanan et al., 2007). Even in chronic rotenone-based Drosophila model system, melatonin alleviates both locomotor impairment and the dopaminergic neuronal loss (Coulom and Birman, 2004). Although melatonin does not directly alter free calcium ion concentration or rotenone-induced inhibition of mitochondrial complex I, it is known to inhibit calcium ion and rotenone combined-induced oxidative stress in isolated rat brain mitochondria (Sousa and Castilho, 2005).

1.8.2.4. Melatonin and methampetamine

The protective effects of melatonin on methamphetamine induced changes in the nigrostriatal system have been widely studied (Imam et al., 2001; Nopparat et al., 2010; Virmani et al. 2002). Methamphetamine-induced increase in free-radical formation owing to incomplete oxidative phosphorylation and mitochondrial damage leading to a failure of cellular energy metabolism followed by a secondary excitotoxicity could be regulated by melatonin (Virmani et al., 2002). It is found that methamphetamine-induced biosynthesis of the ROS, peroxynitrite, which leads to the striatal dopamine depletion, is significantly reduced by melatonin (Imam et al., 2001). Amphetamine-induced changes in α-synuclein, phosphorylated TH and mitochondrial complex I proteins are significantly reversed by
melatonin (Klongpanichapak et al., 2008). Melatonin is reported to rescue methamphetamine-induced depletion of the striatal dopamine and DAT binding sites (Itzhak et al., 1998).

1.8.2.5. Melatonin and MB and PQ

Melatonin prevents the dopaminergic neurodegeneration by inhibiting free radical generation, neuroinflammation and apoptosis induced by MB + PQ possibly in the same fashion as in the case of MPTP. Since MB and PQ combined model is a relatively newer animal model of PD, the studies on the effect of melatonin against this model system are limited. Melatonin and its metabolite N(1)-acetyl-5-methoxykynuramine inhibit the expression and activity of the mitochondrial inducible nitric oxide synthase (iNOS) and prevent mitochondrial failure (Tapias et al., 2009) in many PD models (Fig. 1.3). Melatonin is effective in combating PQ-induced oxidative stress in Drosophila (Bonilla et al., 2006). Melatonin is involved in the recycling of reduced nicotinamide adenine dinucleotide (NADH) and defends against PQ-induced NADH depletion under oxidative stress (Tan et al., 2005). Melatonin checks PQ-mediated DNA damage and genotoxicity by foraging the hydroxyl and other free radicals (Melchiorri et al., 1998; Yamamoto and Mohanan, 2001). Similarly, melatonin reduces PQ-induced mortality in rats and increases the lethal dose 50 (Melchiorri et al., 1996). Melatonin is found to inhibit the MB-induced α-synuclein aggregation and mitochondrial dysfunctions and neurodegeneration at a night time physiological blood concentration (Ishido, 2007). Despite little knowledge about the applications of melatonin against MB + PQ-induced toxicity, it can be inferred from the studies conducted so far that melatonin could be equally effective in this model system as reported in 6-OHDA and MPTP.
1.8.3. Melatonin and possible explanations of the neuroprotective potential

Free-radical generation, mitochondrial pore formation and oxidative stress-induced neuronal cell death are critical in PD and any substance which prevents opening of mitochondrial pores and preserves the mitochondrial function may act as a neuroprotective agent (Bachurin et al., 2003). Melatonin reduces neuroinflammation, oxidative stress and cell adhesion and restores mitochondrial function (Esposito and Cuzzocrea, 2010). It also acts as an important component of the brain’s antioxidant defence system against catecholamine auto-oxidation and protects against the consequent dopaminergic neurodegeneration (Miller et al., 1996). This idea gained momentum from the fact that pineal gland meets the criteria of the neuroendocrine system and dysfunction of the pineal gland may be associated with the pathophysiology and clinical manifestations of PD (Sandyk, 1990a). Although initially it was considered that melatonin offers neuroprotection by restoring its level or by improving the restoration of the expression of TH by reducing its oxidation (Mayo et al., 2005), several theories have been documented till date.

1.8.3.1. Inhibition of dopamine release

An integrated relationship between dopamine and melatonin is essential for normal physiology and any imbalance between the two may lead to PD (Willis, 2008). But as the physiological levels of melatonin decrease with age, therefore, its importance in the total antioxidative defense capacity of an organism is being investigated (Reiter et al., 1997). Melatonin inhibits the release of dopamine in the striatum and limbic system and decreases the blinking rate in PD patients, suggesting a functional link among blink rate, melatonin secretion and the striatal dopaminergic functions (Sandyk, 1990b). PD may show sleep related symptoms and a significant improvement in the subjective sleep
disturbance by melatonin suggests its relevance in PD (Catala et al., 1997; Dowling et al., 2005).

Figure 1.3. Effect of melatonin on major events involved in pesticide-induced apoptosis

1.8.3.2. Antioxidant and free-radical scavenger

As PD imprints long-lasting physiological and pathological permanent effects on the pathogenic proteins, proteasomal machinery, mitochondrial physiology, metabolism, permeability and viability and cellular integrity and apoptosis, therefore, anti-oxidants may help in the amelioration of the symptomatic features of PD. Melatonin, reported to be a potent free-radical scavenger, forages a variety of reactive oxygen and nitrogen species, including hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide and peroxynitrite anion. Furthermore, it can improve the cell survival and functions by
stimulating antioxidant enzymes, increasing the efficiency of the electron transport chain and promoting ATP synthesis (Leon et al., 2004). Even the pharmacological levels of melatonin are used to combat oxygen and lipid peroxides induced toxicities (Mayo et al., 2005). It is several times more potent than vitamin C and E in protecting tissues from oxidative injury when compared at an equivalent dosage (Tan et al. 2002). Melatonin, alone or in combination with deprenyl, defends against the dopaminergic neurodegeneration, as they suppress the hydroxyl radical formation during dopamine autoxidation in vitro (Khaldy et al. 2000; Khaldy et al. 2003).

Melatonin regulates circadian modulation of Na+/K+-ATPase and Na+/H+ exchanger owing to its antioxidative and membrane fluidity modulating properties (Chakravarty and Rizvi, 2011a). Melatonin-mediated rhythmic modulation of malondialdehyde and intracellular glutathione contents during day and night emphasizes the role of melatonin as an antioxidant and its function against oxidative stress (Chakravarty and Rizvi, 2011b).

**1.8.3.3. Mitochondrial modulator**

As inhibition of mitochondrial complexes I to IV have been implicated in the pathogenesis of PD, therefore, a modulator of any of these, if not all, may be used to protect the dopaminergic neurons from damage. Melatonin preserves mitochondrial homeostasis, enhances mitochondrial glutathione level and preserves proton potential and ATP synthesis by stimulating complex I and IV activities (Srinivasan et al., 2005). Melatonin increases the activity of the complex I and complex IV and improves mitochondrial respiration, increases ATP synthesis under normal and stressful conditions and also ameliorates the harmful reduction in the mitochondrial membrane potential that may trigger mitochondrial transition pore opening and the apoptotic cascade (Leon et al., 2005). Melatonin protects against the deficits in the mitochondrial complexes leading to
free-radical mechanisms, both directly via ROS production and indirectly by decreased ATP synthesis and energy failure (Dabbeni-Sala et al., 2001).

1.8.3.4. Anti-apoptotic molecule

Apoptosis is one of the major critical events in the pathophysiology of neurodegenerative disease especially PD. Melatonin inhibits the apoptotic gene and protein expression patterns similar to many neuroprotective agents (Weinreb et al., 2003). Melatonin also displays an extremely low index of mortality and even its high concentration does not significantly affect the expression of the mitochondrial Bcl-2 family members, Bcl-2 and Bax (Bachurin et al., 2003). Melatonin also inhibits oxidative stress-induced NF-kB (nuclear factor kappa-light-chain enhancer of activated B cells) activation, one of the main molecular hallmarks of the apoptotic events in PD (Lezoualch et al., 1998).

Extracellular signal-regulated kinase (ERK) signaling is involved in the transcription of various genes responsible for the cell survival. Melatonin increases the phosphorylation of ERK and activates the mitogen-activated protein kinase pathway in gonadotropin-releasing hormone secreting neuronal cell line (GT1–7 cells) (Roy and Belsham, 2002). Similarly, Ak transforming serine/threonine kinase (Akt) is one of the most important mediators of growth factor-induced cell survival and regulates cell proliferation, apoptosis and cell-cycle progression (Kim and Chung, 2002). Melatonin is known to induce Akt phosphorylation via melatonin receptor- and phosphatidyl inositol-3-phosphate kinase-independent pathways in the primary astrocytes (Kong et al., 2008) and defends against neuronal death in the hippocampus (Lee et al., 2006) and cerebral ischemia (Kilic et al., 2005).
1.8.3.5. Growth factor promoter

Melatonin attenuates the compensatory contralateral increase in the striatal GDNF expression, supporting a physiological role for melatonin in correcting the expression of growth factors, which is normally defective in PD (Sharma et al., 2006). Melatonin induces GDNF and melatonin receptor expressions, which supports a functional role for the MT1 receptor, as they are co-localized and their interaction possibly offers neuroprotection against PD (Niles et al., 2004). An induction of GDNF mRNA by melatonin also shows how it maintains the nigrostriatal dopaminergic integrity, as GDNF is essential to protect the neurons (Armstrong and Niles, 2002).

1.9. Silymarin

Silymarin \[2-(2, 3-Dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-1, 4-benzodioxin-6-yl)-2, 3-dihydro-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one]\] possess flavonolignan phenolics ring structure and antioxidative property (Jain et al., 2011). It is currently available as standard mixture of silibinin, isosilybin, silydianin, silychristin and a few minor ingredients (Lee et al., 2007b; Saller et al., 2007). Silymarin is a flavonoid complex extracted from the fruit of *Silybum marianum* [(L.) Gaertn. (Carduus marianus L., Asteraceae)], which is commonly known as milk thistle. This is the native plant of Mediterranean but grows throughout Europe, North America (Luper, 1998; Pepping, 1999), India, China, South America, Africa and Australia. Silymarin, an herbal drug, is used traditionally by the physicians for the treatment of many diseases and disorders. Moreover it is nontoxic even at a very high dose (Jacobs et al., 2002; Saller et al., 2001; Saller et al., 2007).

Silymarin and its isomeric forms silibinin and isosilibinin, offer protection against chemically induced inflammation, hepatotoxicity and cancer (Upadhyay et al., 2007;
Chapter 1  Review of literature

Upadhyay et al., 2010; Wang et al., 2008). Silymarin prevents epithelial, prostate, bladder, lung, ovarian and breast cancers (Shalan et al., 2005; Kaur and Agarwal, 2007; Gazak et al., 2007; Svobodova et al., 2007). Silymarin, a powerful hepatoprotective agent, possess antioxidant and anti-inflammatory properties, encounters against apoptosis and free radical generation. Silymarin is reported to protect against mitochondrial dysfunction by the prevention of mitochondrial proton leakage (Serviddio et al., 2010). Silymarin also modulates the activity of detoxification enzymes, such as SOD, catalase and GPx. Silymarin reduces the expression of pro-inflammatory cytokines and pro-apoptotic proteins (Wang et al., 2002; Kim et al., 2009).

Silymarin, crosses the BBB, enters the brain (Nencini et al., 2007) and offers neuroprotection against PD (Baluchnejadmojarad et al., 2010). Although, little is known about the neuroprotective properties of silymarin, its antioxidant and anti-inflammatory properties could be main contributory factors for its efficacy against neurodegenerative disorders. Silymarin reduces the lipid peroxidation, iNOS expression and nitrite content in \textit{in vitro} and \textit{in vivo} (Wang et al., 2011; Wang et al., 2002; Chtourou et al., 2010; Galhardi et al., 2009).

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{silymarin_structure.png}
\caption{Chemical structure of silymarin (http://www.chemblink.com)}
\end{figure}
Silymarin, crosses the BBB, enters the brain (Nencini et al., 2007) and offers neuroprotection against PD (Baluchnejadmojarad et al., 2010). Although, little is known about the neuroprotective properties of silymarin, its antioxidant and anti-inflammatory properties could be main contributory factors for its efficacy against neurodegenerative disorders. Silymarin reduces the lipid peroxidation, iNOS expression and nitrite content in \textit{in vitro} and \textit{in vivo} (Wang et al., 2011; Wang et al., 2002; Chtourou et al., 2010; Galhardi et al., 2009).

1.9.1. Silymarin and toxin-induced model of PD

1.9.1.1. Metals

Mn exposure could lead to PD by increasing the oxidative stress. Silymarin reduces neurotoxicity induced by manganese chloride \textit{in vivo} (cerebral cortex of rats) and \textit{in vitro} (Neuro2a cells) by decreasing of the enzymatic (SOD, catalase, GPx) and non-enzymatic (glutathione, non protein thiols, Vitamin C) antioxidants (Chtourou et al., 2010).

1.9.1.2. LPS

Silymarin shows neuroprotective effect against LPS-induced neurotoxicity in mesencephalic mixed neuron-glia cultures by reducing the NF-\(\kappa\)B, nitrite and iNOS expressions and thereby the nigrostriatal dopaminergic neurons degeneration (Wang et al., 2002). Silymarin also reduces the oxidative stress by inhibiting the level of lipid peroxidation and oxidized protein in cortical region of aged rat brain (Galhardi et al., 2009).

1.9.1.3. 6-OHDA

Silymarin reduces neurotoxicity by inhibiting oxidative stress through estrogenic pathway and reduces the number of apomorphine-induced unilateral rotations in rats
(Baluchnejadmoharad et al., 2010). Effect of silymarin on TH-positive cells, dopamine content, apoptosis and inflammation in 6-OHDA-induced PD are not yet reported (Bernstein et al., 2011; Koprich et al., 2008).

1.9.1.4. MPTP and rotenone

Although, MPTP is used from the last 30 years and rotenone maximally mimics PD pathology, the effects of silymarin on MPTP and rotenone-induced PD have not been looked into.

1.9.1.5. PQ and MB

PQ and MB induce the formation of ROS by the inhibition of mitochondrial complex I and III, respectively. MB and PQ both are well known to induce dopaminergic neurodegeneration in the SNpc region of the midbrain (Thiruchelvam et al., 2000b). The effects of silymarin on MB + PQ-induced PD phenotype are not reported before this study.

1.9.1.6. Methamphetamine

Silibinin, a component of silymarin, is reported to protect against methamphetamine-induced neurotoxicity in the mice (Lu et al., 2010). Silymarin also increases the dopamine content in the mesocortical pathway and serotonin level in the hippocampus induced by the repetitive treatment of methamphetamine (Lu et al., 2010).

1.9.2. Silymarin and neuroprotection

Silymarin reduces the expression of amyloid β-protein fibril formation in both in vitro and in vivo (Murata et al., 2010). It reduces the cerebral ischemia induced brain infarction and improves neurological deficits in stroke models. silymarin reduces the increased level of lipid peroxidation, protein nitrosylation and oxidative stress, NF-κB, signal transducer and
activator of transcription (Stat)-1, IL-1β, TNF-α, iNOS and Cox2 during cerebral ischemia (Hou et al., 2010).

Silibinin, which is component of silymarin, protects from autophagy and cellular oxidoreductase activities via inhibiting NF-κB activation and ROS production against D-galactose-induced senescence (Wang et al., 2011). Silibinin significantly attenuates the decrease in the dopamine in the prefrontal cortex and serotonin in hippocampus and improves cognitive impairment, which are caused by methamphetamine-induced neurotoxicity (Lu et al., 2010).

![Image of a diagram showing the effect of silymarin on major events involved in pesticides-induced apoptosis]

**Figure 1.5.** Effect of silymarin on major events involved in pesticides-induced apoptosis
1.9.3. Silymarin and possible mechanism of neuroprotection

Environmental toxins and infections can provoke the excessive production of ROS, which may lead to neuronal damage by interacting with DNA, proteins and lipids (Storey, 1996; Davies, 2000). Silymarin reduces the oxidative stress, inflammation and apoptosis and improves the mitochondrial function (Nencini et al., 2007; Galhardi et al., 2009; Hou et al., 2010; Manna et al., 1999).

1.9.3.1. Silymarin as an antioxidant

Age-related brain disorders and neurodegenerative diseases are caused by an increased oxidative stress. It is interesting to explore new compounds, which improve cognitive performance and offers neuroprotection through antioxidant activity (Metodiewa and Koska, 2000). Free radicals create oxidative stress within the cell and the imbalance between ROS production and antioxidant defence system could be harmful to proteins (undergo changes in amino acids), lipids (disruption in the membrane fluidity) and DNA (breaking or cross-linking with another DNA chain) (Storey, 1996; Davies, 2000).

Silymarin reduces lipid peroxidation, iNOS and Cox2 levels in the cerebral ischemia/reperfusion (Hou et al., 2010). Silymarin reduces neurotoxicity induced by manganese chloride and acetaminophen by restoring the level of glutathione, ascorbic acid and SOD and decreases the malondialdehyde and oxidized glutathione levels (Chtourou et al., 2010; Nencini et al., 2007; Galhardi et al., 2009).

1.9.3.2. Silymarin and anti-inflammation

Silymarin has been reported as an anti-inflammatory compound and inhibits the expression of NF-κB, IL-1β, TNF-α and iNOS, which are induced during cerebral ischemia (Hou et al., 2010). Silymarin also reduces the expression of NF-κB, nitrite and
iNOS against LPS-induced neurotoxicity in mesencephalic mixed neuron-glia cultures and offers neuroprotection by rescuing the dopaminergic neurons (Wang et al., 2002).

1.9.3.3. Silymarin and anti-apoptosis

Silymarin decreases oxidative stress and protects cells against apoptosis (Manna et al., 1999). Milk thistle extract prevents the apoptosis induced by oxidative stress in primary hippocampal neurons and neurite outgrowth and trophic factor deprivation in PC-12 cells (Kittur et al., 2002). Silibinin reduces the apoptotic effect induced by sodium nitroprusside in PC12 cells by inhibiting the autophagic pathway, which is involved in Ras/Phosphoinositide 3-kinase/ NF-κB (Ras/PI3K/NF-κB) and non-autophagic pathway that involved in Ras/Raf/Mitogen-activated protein kinase kinase/ERK (Ras/Raf/MEK/ERK) pathway (Liu et al., 2011).

Lack of toxicity even at high doses, ability to cross the BBB, antioxidant, anti-inflammatory and anti-apoptotic nature of silymarin prompted the researcher to investigate the effects of silymarin against biochemical and molecular indices of MB + PQ-induced PD phenotype in mice.