4. REVIEW OF LITERATURE

4.1 PD PATHOPHYSIOLOGY

The pathophysiology of idiopathic Parkinson’s disease (PD) was found to be due to progressive loss of dopaminergic neurons from substantia nigra pars compacta (SNpc) of the mid brain, which contains neurotransmitter, dopamine (3,4-dihydroxy phenyl ethylamine/ 3-hydroxytyramine-DA). Since, these neurons project their axons to the striatum and utilize dopamine as their neurotransmitter; a profound reduction in striatal dopamine represents the primary neurochemical alteration in PD (Bergman and Deuschl 2002; Dauer and Przedborski 2003). In-addition to dopamine depletion there is concomitant loss of dopamine metabolites homovanillic acid (HVA) and 3, 4- dihydroxyphenylacetate (DOPAC) and an increase in dopamine receptor sites were reported in PD brain (Hornykiewicz, 1966; Lee et al. 1978). Furthermore, cumulative evidences suggest that in PD pathogenesis, there may also be a deficiency of other neurotransmitters including, norepinephrine (NE) and serotonin (5-HT) which may contribute to some of the secondary symptoms as depression in the PD progression (Nayyar et al. 2009). Furthermore, the abnormal aggregation of α-synuclein forms Lewy bodies (LBs) in surviving dopaminergic neurons is the pathological hallmark of the PD. LBs are rich in neuromelanin and present in the basal ganglia, brain stem, spinal cord, and sympathetic ganglia (Spillantini et al. 1997).
Figure 4.1: Schematic diagram showing the nigrostriatal dopaminergic pathway. A cross-section of human brain showed the caudate and putamen zone of the striatum. A section through the midbrain shows the *substantia nigra*. Nigrostriatal dopaminergic pathway (from substantia nigra to nerve terminals represented as dark line, starting from cell body and ending to nerve terminals), whose cell bodies are located in the SN, send projections that terminate and release dopamine in the striatum. With the degeneration of the dopaminergic pathway, there is a progressive drop in dopamine release into the striatum. Striatal dopamine deficiency, in turn, results in complex changes in the brain's motor circuitry and causes the motor deficits characteristic hallmark of Parkinson's disease (Dinis-Oliveira et al. 2006).

### 4.2 EPIDEMIOLOGY OF PD

PD poses a significant public health burden, which is likely to increase in the coming years; recent epidemiological reports suggested that the prevalence of PD is in between 0.1 to 0.3 % in general population and between 1-2% in persons 65 years old and above (Pedersen et al 2009; Weintraub et al. 2008). Moreover, WHO report (2004) speculated that neurodegenerative disorders including Alzheimer’s disease and PD is expected to surpass cancer as the second most common cause of death by the year 2040. The direct and indirect costs for the care of PD patients, including cost of drug treatment (about US$ 1100 million worldwide) can be substantial (WHO, 2004). Furthermore, improving economy and health care in developing countries including India, has increased the life expectancy of the elderly population; subsequently increase in PD cases (Muthane et al. 2007).
4.3 ETIOLOGY OF PD

The etiology of PD is not well understood but likely to involve both genetic and environmental factors. It is estimated that 90-95% of PD cases are idiopathic/sporadic with the remaining cases arising from genetic mutations (Olanow and Tatton 1999; Daur and Przedborski 2003). Despite numerous emerging data from pre-clinical and clinical studies, the cause of idiopathic PD remains enigmatic. Genetic studies have identified numerous mutations that cause inherited form of PD and pathological studies with post-mortem PD brains have suggested several key cellular mechanisms like, oxidative stress, mitochondrial dysfunction, and proteasome dysfunction are responsible for the disease pathogenesis and progression. In-addition, epidemiological studies have highlighted the connection of environmental agents, such as pesticides/herbicides in the development of PD. The etiological factors are listed herein.

4.3.1 Environmental factors behind PD progression

The etiology of idiopathic PD remains enigmatic. Growing evidences suggest that the environmental factors, rural living and consumption of well water, attributes major risk factor in PD progression (Tanner and Langston, 1990). Moreover, epidemiological studies have implicated pesticide exposure as a potential risk factor for PD (Priyadarshi et al. 2001; Di Monte et al. 2002). In this context, several reports suggest that paraquat administration induces up-regulation and aggregation of α-synuclein, providing an intriguing model of interactions between protein and environmental toxicants (Manning-Bog et al. 2002; Thiruchelvam et al. 2000). Pertaining to it, several reports suggested that potent environmental hazards as herbicides, pesticides and heavy metals are impetuous factors in PD development.

4.3.2 Genetic factors associated with PD

Majority of PD cases are appears to be sporadic in nature; however low prevalence of PD (<10%) cases are genetically linked which intended the high probability of developing familial
PD (Gasser, 2001). Bonifati and co-workers (2003) have reported that primarily, there are four genes found to be closely associated to PD pathogenesis. The first PD gene, PARK1, was identified as mutant forms of the gene encoding the presynaptic protein α-synuclein. Further, reports showed that the second gene is an alanine to threonine substitution codon 53 (A53T) in α-synuclein; the third PD gene, PARK7, results from mutations in DJ-1 and suggested that mutations in α-synuclein, parkin, and DJ-1 definitely causes PD. Polymeropoulos et al (1997) has reported that mutation (PARK5) in the gene encoding ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in autosomal dominant PD cases. Therefore, it is evident that mutation in particular genes are strongly associated with PD pathogenesis.

4.4 NEUROCHEMICAL ALTERATIONS IN PD

It is evident that catecholamines [dopamine (DA), noradrenaline (NA) and adrenaline (A)] are derived from a common precursor-tyrosine. In DA biosynthesis, tyrosine is converted to 3, 4-dihydroxy-L-phenylalanine (L-DOPA) in the presence of enzyme tyrosine hydroxylase (TH), which is a rate-limiting enzyme for the catecholamine biosynthesis. Once L-DOPA synthesized, further it is de-carboxylated to DA by the enzyme aromatic L-amino acid decarboxylase (AADC). Since, AADC is active in both the peripheral and the central nervous system (CNS), DA can be produced in and outside the brain. However, since DA cannot cross the blood brain barrier (BBB), it needs to be synthesized from L-DOPA within the brain. NA is produced from DA by the action of dopamine p-hydroxylase (DBH). Moreover, DA can also be transported inside noradrenergic terminals, where it is converted to NA. Released catecholamine can be inactivated by reuptake into the pre-synaptic neuron by specialized membrane monoamine transporters, including the DA transporter (DAT) and NA transporter (NET) and also can also be inactivated by turnover (metabolism); turnover of monoamines is governed through enzyme monoamine oxidase-A and B activity (MAO-A and B).
4.4.1 Noradrenergic neuropathology of PD

The largest noradrenergic system in the brain is the one ascending from the locus coeruleus (LC), which has extensive projections into the entire neocortex and the limbic forebrain (amygdala, septum, hippocampus). Although very low concentrations of NA occur in the striatum but higher level of NE is available in SNpc (Rabey and Burns, 2002), noradrenergic neurons directly innervate the striatum. It is evident that stimulation of the LC facilitates firing of SNpc neurons, while administration of a noradrenergic receptor antagonist attenuates this. These observations are supported with the finding that both lesions of the LC or chronic NA depletion decreases striatal DA release and result in the compensatory up-regulation of striatal D2 receptors. Dopamine beta hydroxylase (DBH), NA and the NET can be detected in the midbrain and striatum, indicating the intricate role of NA in the basal ganglia. As mentioned before, in metabolism, DA is required for NA synthesis indicating their strong relationship and mutual importance in PD. Rommelfanger and co-workers (2007) have reported that NA has important functions in the nigrostriatal dopaminergic system and the degree of cell loss in the LC has been estimated to be around 70% in advanced PD, similar to the degree of cell loss in the SN. Subsequent loss of NA occurs in all layers of the cerebral cortex and occurs mainly in the motor, premotor and the supplementary motor cortex, the SNpc and the hippocampus. Moreover, postmortem studies of PD brains revealed inverse correlations between DA loss and NA content in several brain regions, including the striatum, suggesting a possible protective function of NA in PD (Tong et al. 2006). The involvement of NA and a possible protective function has also been indicated in animal models of PD (Mavridis et al. 1991; Bing et al. 1994; Rommelfanger et al. 2007). These studies suggested that the LC is an endogenous modulator of the toxic threshold for DA neurons in different animal species, and that it may have protective function against nigrostriatal neurotoxicity (Gesi et al. 2000). Collectively from these observations it can be
suggested that damage to the noradrenergic system by itself can provoke parkinsonian symptoms, by disrupting basal ganglia functioning. Interestingly from the literature survey we have noticed that role of NA in MPTP neurotoxicity was ignored or unreported. As NA plays intriguing role in motor coordination, it should receive much more attention in the animal studies. Besides role of NA in motor behaviour, it could have important effects on non-motor behaviour, including cognition (Arnsten and Goldman-Rakic, 1985) and mood (Rabey and Burns, 2002). Taken together, it can be assumed that PD should be consider as a combined DA-NA disorder and an animal model that target both systems simultaneously would thus hold great importance. Keeping these points in view, the present study is an effort to investigate correlation between NA-DA and its intricate association with MPTP model.

4.4.2 Serotonergic neuropathology of PD

In PD, mood disturbances such as depression and anxiety are extremely common. Anxiety and depression have also been associated with an increased risk of later development of PD (Schuurman et al. 2002; Shiba et al. 2000). The pathophysiology of mood disturbances in PD remains unclear. The serotonergic dysfunction has been postulated to involve in mood disorders in non-PD cases. The raphe nuclei, as well as hippocampus and prefrontal cortex, appear to be the primary sites affected (Drevets et al. 2007; Groenewegen et al. 2000) in mood disorder patients. The major serotonergic system of the brain is an ascending pathway from mesencephalic raphe nuclei to cerebral cortex. In contrast to NA, the highest concentrations of 5HT and its metabolite (5-HIAA) are found in the SN, striatum, amygdala, and spinal cord, while the comparatively low levels are observed in the neocortex and the hippocampus. Interestingly, dopaminergic transmission in both the SN and the striatum is known to be under the inhibitory control of ascending serotonergic systems from the Raphe. Despite these richer innervations of the nigrostriatal system by 5-HT than NA and its interaction with the dopaminergic system, this non-
catecholaminergic monoamine appears to have less close ties with PD neuropathology and clinical symptoms (Rabey and Burns, 2002). Melamed and co-workers (1986) have demonstrated that prior destruction of striatal 5-HT neurons did not affect DA depletions produced in the striatum by MPTP-intoxication, suggesting that a similar lack of relevancy of the serotonergic system also applies to experimentally induced parkinsonian syndrome. Nevertheless, increased 5-HIAA/5HT ratios are found in the striatum and frontal cortex of PD patients and MPTP treated mice, suggesting increased 5-HT turnover in these brain regions, which could be a compensatory mechanism (Rozas et al. 1998). Furthermore, compensatory sprouting of serotonergic afferents into the striatum after MPTP intoxication was found in mice and monkeys (Gaspar et al. 1993).

Taken together, present study has been designed to investigate the intricate association between catecholamine and serotonergic neurotransmission in the MPTP-induced mice brain and the effect of withanolides and curcuminoids over these neurochemical alterations.

Besides the considerable understanding of the PD pathogenesis, etiological factors and neurochemical alterations, still the exact mechanism involved in the disease initiation and progression are not well understood. Nevertheless, there have been many advances in the elucidation of the mechanism by which the neurodegeneration of PD leads to its clinical manifestations, the cause of the selective dopaminergic degeneration remains largely unidentified. However, several mechanisms have been postulated behind the disease progression, some of them are discussed herein.

4.5 HYPOTHESIS ATTRIBUTED BEHIND THE PD PATHOGENESIS

Several hypotheses have been put forward and investigated the idiopathic forms of disease, however, no single factor seems to be accountable for idiopathic PD. Rather it seems to be a result of multiple factors and/or intricate cascade of events, to produce the PD pathology. There are several probable factors responsible for PD pathogenesis including exposure to
environmental toxins/occupational hazards (Greenamyre et al. 2003; Sherer et al. 2002), dopamine auto-oxidation and metabolism (Tse, 1976; Graham, 1978), oxidative stress (Sayre et al. 2005; Chinta and Andersen 2008), mitochondrial dysfunction (Greenmyre et al. 2001; Lin and Beal 2006; Schapira 2010;), iron dysregulation (Kaur and Anderson, 2004), proteasome dysfunction (Betarbet et al. 2005), and susceptibility genes (Klein and Schlossmacher, 2006) have been suggested to contribute in idiopathic PD pathogenesis. In addition, some other factors such as perturbed metal homeostasis, oxidative stress and mitochondrial dysfunction are intricately linked in all the pathogenic factors of PD. Interestingly, the cellular pathology is almost similar in both familial and sporadic forms of PD like, impairments in mitochondrial complex I of the electron transport chain, along with resultant increase in oxidative stress markers as well as decreased expression and levels of brain-derived neurotrophic factor (BDNF) (Howells et al. 2000; Jenner and Olanow 1998; Jha et al. 2000). Both mitochondrial and BDNF anomalies are also present in the MPTP induced animal models of PD (Hung and Lee, 1996; Przedborski and Jackson-Lewis, 1998). Some of the intricate mechanisms involved behind the PD progression are enlisted herein.

4.5.1 Mitochondrial dysfunction and associated oxidative stress in PD pathogenesis

The mitochondrion is a double membrane bound organelle found in most eukaryotic cells that generates most of cell’s energy in the form of adenosine triphosphate (ATP), which is essential for normal cell functioning and homeostasis. The production of energy in forms of ATP is the most well-known function of mitochondrion besides its role in signalling, cellular differentiation, control of cell cycle and growth, and apoptosis (McBride et al. 2006). The biochemical cascade occurs at mitochondrion to produce ATP; where glucose is metabolized to pyruvate via glycolysis (takes place at cytoplasm). Further pyruvate is transported into the mitochondria and converted to acetyl-CoA. Similarly, fatty acids are also a source of energy that
can be metabolized to acetyl-CoA via β-oxidation. The acetyl-CoA then undergoes first reaction with oxaloacetate in the citric acid cycle catalysed by citrate synthase. Citrate synthase, a quantitative marker of the mitochondria, is an enzyme encoded by the nuclear DNA and is transported into the mitochondrial matrix after its synthesis in cytoplasm. The reducing equivalents produced as NADH in the citric acid cycle are passed along the first complex of the electron transport chain embedded in the inner mitochondrial membrane, which is part of the process also known as the oxidative phosphorylation. It is evident that during the normal process of oxidative phosphorylation, ROS such as superoxide anions, hydrogen peroxide, hydroxyl radicals, and peroxo radicals can be generated as byproducts in mitochondria (Fig. 4.2). In normal functioning cells, ROS has positive roles such as inducing host defense genes and mobilization of ion transport systems. Similarly, the cellular mechanisms exist to convert these molecules into harmless forms, which involve endogenous antioxidants such as superoxide dismutase (SOD), catalase, and glutathione (GSH). Moreover, protein carbonyl content, marker for oxidative damage of proteins available in brain. In contrast, in diseased condition, ROS attacks amino acid residues, particularly histidine, arginine, lysine, and proline, of the target proteins to produce excessive carbonyl groups, which are the oxidized products and exerts deleterious affect over micro and macro molecules within the cell. Furthermore, nitric oxide (NO), generated by the inducible form of nitric oxide synthase (iNOS) in glial cells or the neuronal form (nNOS), participates in the cascade of events leading to the degeneration of dopamine-containing neurons (Danielson and Andersen, 2008; Sawada et al. 2007; Halliwell, 2002).
Cumulative research findings indicated the direct involvement of mitochondrial complex-I impairment in the PD pathogenesis. It is evident that, mitochondrial complex-I directly involve in disease progression instigates from cytotoxic insult caused due to various potent neurotoxins as rotenone and MPTP in the dopaminergic neuronal cells of substantia nigra. Mechanism involved behind MPTP cytotoxicity is that MPTP readily crosses the blood brain barrier and gets converted to MPP⁺ by the enzyme MAO-B primarily located in glial cells (Fuller et al. 1985). Following activation in the glia, MPP⁺, a substrate for the DA transporter, is selectively taken up into DA nerve terminals where it inhibits complex-I of the mitochondrial electron transport chain, culminating to uncoupling of oxidative phosphorylation and production of ROS (Fonck and Baudry 2001; Shimoke et al. 2003), which further triggers selective DA neurodegeneration, either through direct production of ROS from increased levels of free electrons and/or indirectly through dysregulation of iron (Youdim 2003; Lin and Beal, 2006). The mitochondrial complex I, known as NADH dehydrogenase/NADH: quinone oxidoreductase located in the inner mitochondrial membrane and catalyses the transfer of electrons from NADH to coenzyme Q. The process of electron transfer in complex I results in increased membrane potential with protons...
accumulating in the inter membrane space transported from the matrix. Eventually, complexes III and IV receive the transferred electrons from complexes I and II, and contribute to additional increase in the membrane potential and eventually results in ATP synthesis (Fig. 4.3).

Figure 4.3: Schematic representation of mitochondrial electron transport chain. ETC involved in oxidative phosphorylation. CI and II (Complexes I and II) transport electrons (e−s) generated by the conversion of NADH to NAD+ (CI) or FADH2 to FAD (CII) through Q (ubiquinone), CIII, Cyt c (cytochrome c) and finally CIV, which uses an e− to convert O2 to H2O. During electron transfer, CI, II, and IV pump protons (H+s) from the mitochondrial matrix into the inter membrane space generating a H+ concentration gradient that drives the formation of ATP from ADP by ATP-synthase (Complex V); courtesy, Keane et al. 2011.

It is evident that any damages caused in one of these processes would potentially impair the mitochondrial function and thus energy crisis in the cell. Pertaining to it, several reports suggested that the mitochondrial structural abnormalities and reductions in complex I activity have been associated in PD pathogenesis (Schapira et al. 1990; Haas et al. 1995; Triepels et al. 2001; Keeney et al. 2006). Inhibition of complex I function can cause increased production of reactive oxygen species (ROS) such as superoxide anions that can generate highly reactive hydroxyl radicals and peroxynitrites (Pitkanen and Robinson 1996; Raha, 2000), subsequently these deleterious by-products can damage cellular membranes and organelles. Furthermore, several studies demonstrated that neurotoxins such as MPTP, rotenone, and paraquat can inhibit mitochondrial complex I and disturb the respiratory chain enzymes which leads to impaired
energy metabolism and intracellular ROS production (Bretaud et al. 2004). Moreover, dramatic mitochondrial abnormalities in substantia nigra were observed in MPTP treatment of 12-month old α-synuclein over-expressing transgenic mice (Song et al. 2004). Mitochondrial abnormalities and oxidative stress have also been demonstrated in-vitro from the over-expression of wild type human α-synuclein in cultured cells (Hsu et al. 2000). The results obtained from in-vivo and in-vitro studies link the effects of neurotoxins and over-expression of α-synuclein in the development of mitochondrial dysfunction and PD. During mitochondrial oxidative metabolism, molecular oxygen is reduced to water at complex IV of the ETC. The 1-2% of oxygen that is not reduced at complex IV is reduced non-enzymatically to superoxide (O2.) and H2O2 by electrons that leak from the sites in ETC. In PD brain, there is a site of electron leak in complex I of the ETC. Other studies have also reported a reduced complex-I activity in the platelets (Bindoff et al. 1989) and in skeletal muscle (Blin et al. 1994) in PD patients. Moreover, there are substantial reports suggesting that there has been selective loss of GSH in SN of PD patients. It is evident that GSH depletion potentiates oxidant-induced loss of mitochondrial functions, oxidative stress which triggers, damage to DNA, lipids, and protein (Dexter et al. 1989). Taken together, these observations suggest that mitochondrial dysfunction can compromise DA neuronal viability and responsible for the PD pathogenesis.

4.5.2 Oxidative stress and PD progression

Mounting evidences suggested that oxidative stress is a major contributing factor to PD pathogenesis. It has received more attention in PD because of the oxidative metabolism of dopamine to yield hydrogen peroxide (H2O2) and other reactive oxygen species (ROS) (Olanow, 1993; Anderson 2008; Miller et al. 2008). Several clinical and preclinical studies suggested the involvement of oxygen-free radicals and oxidative stress in the pathogenesis of PD. Among several oxidative stress markers associated with PD, one of the first biochemical changes noted in the disease is a decrease in glutathione levels in the substantia nigra of PD cases (Fukui and
Moraes, 2008). Furthermore, an increase in the levels of the nucleic acid oxidation product 8-hydroxyl-guanosine and lipid peroxidation by-products (MDA, TBARS, 4-HNE) are markers of oxidative stress, is increased in neurons of PD patients, whereas its levels remain lower in surrounding brain regions (Danielson and Andersen, 2008; Halliwell, 2002). In-addition, increased levels of iron and reduction of ferritin concentrations in the SNpc of PD patients has been reported (Dexter et al. 1990; Jellinger et al. 1993; Gotz, et al. 2004). It is evident that the vulnerability of nigral neurons to the toxic insult of ROS is primarily due to the high content of transition metals and iron present in SN (Poirier et al. 1985).

Furthermore, oxidative stress could lead to dopaminergic neurodegeneration under circumstances including, increased dopamine turnover, causes excess peroxide production; excessive enzymatic oxidation of dopamine in the synaptic cleft; a deficiency in glutathione (GSH), thereby diminishing the brain’s capacity to clear $\text{H}_2\text{O}_2$, an increase in reactive iron, which can promote OH formation and decrease in mitochondrial complex-I activity. Most of the toxicity observed following superoxide anion generation is believed to be due to the reaction of the superoxide anion with other ROS such as nitric oxide (NO$^\cdot$). Reaction of the superoxide anion with NO$^\cdot$ produces the potent oxidant peroxynitrite (ONOO$^-$), which has been implicated in various neurotoxic and neuropathological events (Crow and Beckman, 1995). Moreover, it has been also reported that the excessive peroxynitrite triggers the activation of poly ADP-ribose polymerase (PARP) that is a highly energy dependent process and leads to the cleavage of NAD$^+$ into ADP-ribose and nicotinamide. PARP activation rapidly depletes NAD$^+$ stores, thereby impairing mitochondrial function, glycolysis, and ATP synthesis (Ying et al. 2001).

4.5.3 Auto-oxidation of dopamine

Increased level of DA itself is a source of oxidative stress in dopaminergic neurons. It has been postulated that the susceptibility of nigral neurons is in-part due to increased level of
oxidative stress resulting from the catabolism and auto-oxidation of DA (Stokes et al. 1999; Fornstedt et al. 1990). Moreover, DA also auto-oxidizes to the DA quinone by a process that is dramatically increased in the presence of transition metals (copper and iron) (Halliwell and Gutteridge 1984). Furthermore, it has been reported that gene linked to familial PD, α-synuclein and parkin, are both covalently modified by the DA quinone. Quinones interact with bioactive molecules, inducing cytotoxicity in and around the dopaminergic neurons by covalently modifying cysteine residues, inhibiting protein function (Miyazaki et al. 2008). Modifications by DA results in altered function of these proteins in-vitro and is suggested to occur in-vivo in the case of parkin (Conway, 2001; LaVoie, 2005). The DA quinone can also react with ROS, reactive nitrogen species (RNS), and with glutathione exacerbating the cellular homeostasis. The reaction of DA with GSH can indirectly result in increased level of oxidative stress by depleting a critical cellular defence mechanism against ROS and RNS (Conway 2001; LaVoie 1999). In-addition, DA quinones also catalyses the production of hydrogen peroxide and the highly reactive superoxide ion (O2·) (Tse et al. 1976; Graham, 1978) both of which can indirectly or directly modify protein and lipid function. Several reports are available suggesting that, DA can also induce apoptosis when applied to cell culture studies (Hoyt et al.1997; Walkinshaw and Waters 1995), an oxidative process that can be prevented by co-administration of anti-oxidant compounds (Hastings et al.1996; Abinovic et al. 2000). The catabolism of DA by MAO-B triggers the production of hydrogen peroxide and ROS, further potentiating the oxidative burden in dopaminergic neurons (Stokes et al. 1999; Chinta et al. 2005). The overall outcome of DA auto-oxidation/metabolism is depletion of cellular antioxidant defence mechanism with a concomitant increase in ROS production, resulting to impaired function of protein, lipids, DNA and mitochondrial dysfunction.
4.5.4 Inflammatory cascades in PD pathogenesis

Inflammation induced neuronal cell death mediated by activated microglia has been implicated in the neurodegenerative diseases including PD, Alzheimer's disease, multiple sclerosis, and stroke. Clinical and pre-clinical findings demonstrated higher microglial expression within the substantia nigra region of PD cases as compared to normal controls (Imamura et al. 2003; Barcia et al. 2004; Ouchi et al. 2005; Sawada et al. 2007). Cumulative evidences suggested microglial mediated cell death of NSDA neurons likely occurs in humans and in several animal models of PD. As intriguing cascade were involved in the inflammatory response, primarily it is apparent that microglial and astroglial cell activation is the first event in the PD progression. Microglial cells are the resident immune cells and constitute about 15% of all cells of the brain that are derived from amyloid lineage and are similar to macrophage cells found outside of the central nervous system (Rock et al. 2004; Kim et al. 2000). Microglial cells serve as an immune surveillance in the central nervous system in their resting state and have a complex branched morphology. It has been reported that activation of microglial cells leads to a rapid transformation from a ramified shape to an amoeboid circular shape in toxic insult or in diseased condition.
Moreover, microglial activation also results in a significant increase in the number of cell membrane receptors involved in the inflammatory response and an increase in the release of inflammatory cytokines and free radical species (Rock et al. 2004; Vilhardt 2005). Concomitantly, activated microglia releases pro-inflammatory mediators as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), prostaglandins, chemokine, ROS and reactive nitrogen species in the diseased condition (Hanisch, 2002).

4.5.5 Protein aggregation in PD

One of the pathological features of PD is aggregation of α- synuclein and subsequent formation of intra cytoplasmic large protein aggregates called Lewy Bodies (LBs). Post mortem reports of PD patients have revealed the presence of LBs in all dead and dying dopaminergic neurons of SN. The main protein in LB is α-synuclein, a 140 amino-acid-long neuronal protein which is found in the highly ubiquitinated form (Wood-Kaczmar et al. 2006). Lee and co-workers (2006) have reported that α-synuclein fibrillization could be enhanced by post-translational modifications, oxidative and nitrative stress, and interaction with metals, such as aluminium, copper, and iron. It is apparent that the accumulation of unfolded or misfolded proteins by insufficient clearance represents a major stress to the cells which further activates the cellular chaperones to assist in refolding of proteins. Moreover, it is also evident that cells use an efficient degradation process to eliminate proteins with unwanted conformations; primarily through the ubiquitin/proteasome pathway (Conway et al. 2001; Hershko and Ciechanover 1998; Mori et al. 2000). Further, the ubiquitin/proteasome pathway involves tagging of misfolded proteins with a small peptide ubiquitin. The defect in the capacity of ubiquitin proteasome system to clear damaged ubiquitinated proteins causes an accumulation of misfolded proteins which aggregate and form Lewy Bodies. Pertaining to it, cumulative evidences suggest that defect in ubiquitin proteosome system is a major factor behind the etiopathogenesis of PD (McNaught et al. 2003; Betarbet et al. 2005).
4.5.6 Excitotoxicity events in PD

Excitotoxicity involves toxicity resulting from increased glutamate formation and is an important contributing factor to the pathogenesis of Alzheimer’s disease and Parkinson’s disease (Beal 1995; Hantraye et al. 1996; Braidy et al. 2010). It has been reported that, SNpc dopaminergic neurons are rich in glutamate receptors that receive extensive glutamate from the cortex and the STN, and demonstrate a pattern of burst firing in response to exogenously administered glutamate (Johnson et al. 1992). Glutamatergic pathways project from basal ganglia to thalamus and back to cortex. There are three types of glutamatergic receptors, i) NMDA, ii) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and iii) kainate. It is evident that the excitatory amino acids become more cytotoxic if released in excess, or if the mechanism of inactivation is impaired. Another excitotoxic mechanism is due to a reduction in energy metabolism due to a defect in mitochondrial function, resulting in loss of the ATP-dependent Mg-blockade of NMDA receptors, causing physiological concentrations of glutamate to mediate a calcium influx into the cell. Excitotoxic damage is also mediated at least in-part via NO. A glutamate-mediated rise in cytosolic calcium level results in activation of nitric oxide synthase (NOS) which triggers increased NO production. NO reacts with superoxide radical to form peroxynitrite and hydroxyl radical, both are powerful oxidizing agents (Dawson et al. 1993). There being cumulative evidences which suggest that excitotoxicity plays a crucial role through aforementioned cascades in the PD pathogenesis (Braidy et al. 2010).

4.5.7 Calcium and PD pathogenesis

Hirsch and co-workers (1997) have suggested that in pathogenesis of idiopathic PD, an altered level of calcium-dependent proteases has been implicated to play a role in differential vulnerability of the dopaminergic neurons. Moreover, Ross et al (2001) observed an increased activity of calcium-stimulated phospholipase A2 in putamen, which was inferred to be due to
either decreased dopaminergic input in striatum or to a DA nerve terminal degenerative process. Furthermore, overexpression of calpain-II, a calcium-dependent protease has been reported in the parkinsonian SN, suggesting a rise in intracellular calcium concentrations is involved in the mechanism leading to the dopaminergic cell death in PD (Mouatt-Prigent et al. 1996). Cumulative research findings suggested that the involvement of different calcium-binding proteins in idiopathic PD has been extensively explored to assess the role of these proteins in buffering calcium load. Consistent with the hypothesis of buffering calcium load in PD condition, Mohanakumar et al (2002) have demonstrated that MPTP causes a rapid depletion of mitochondrial calcium pool followed by a marked and sustained elevation of cytosolic free calcium. Recently, it has been reported that neurons that contain calcium-binding proteins appear to be less vulnerable than the neurons that lack it, suggesting that calcium-binding proteins might protect these neurons from degeneration by preventing excessive increase in cytosolic calcium concentrations (Surmeier, 2007). Furthermore, calcium channel antagonists such as nimodipine and flunarizine have been reported to improve nigral graft survival in neural transplantation studies and PD patients (Brundin et al. 2000; Robinson, 2010). Moreover, evidences for the involvement of calcium in PD pathogenesis is obtained from patch-clamp studies on dorsal root ganglia neurons, where MPP+ has been shown to have a profound and irreversible effect of total as well as K⁺-activated currents (Mohanakumar et al. 2002). Similarly, Samantaray et al (2003) have reported that calcium antagonist nicardipine protects against MPTP-induced dopamine depletion in the striatum.

4.5.8 Apoptosis

Apoptosis or programmed cell death, characterized by marked cell shrinkage, fragmentation of nuclear DNA by endonucleases, and chromatin condensation with the formation of nuclear or “apoptotic” bodies has long been inferred to confer the slow, progressive death of nigral neurons in PD (Anglade et al. 1997; Novikova et al. 2006; Perier et al. 2012). In contrast,
necrosis is a rapid form of cell death that is characterized by massive ionic influx, cellular swelling with disruption of subcellular organelles by rupture of plasma membrane, but without extensive damage to nuclear DNA; necrosis also mediates dopaminergic cell death in acute toxic insult model (Pu and Weidong, 2008). In neuronal cell apoptosis, the family of Bax/Bcl-2, interleukin 1β converting enzyme (ICE) and caspases have received particular attention and several research findings demonstrated increased expression of Bax or caspases promotes apoptosis, whereas increased expression of Bcl-2 or Bcl-xL, promotes cell survival. Schlingensiepen et al (1994) have demonstrated that c-jun is transiently expressed in the early stages of neuronal apoptosis. Antisense oligonucleotides that block the translation of c-jun mRNA and overexpression of a negative c-jun mutant reduced apoptosis, and facilitated neuronal survival. In contrast, overexpression of c-jun increases apoptosis (Ham et al. 1995). There are reports that suggest p53 gene knock-out transgenic mice are resistant to MPTP neurotoxicity (Trimmer et al. 1996) and Bcl-2 overexpression protected catecholaminergic cells against MPTP neurotoxicity (Yang et al. 1998). Viswanath et al (2001) have noticed that MPTP-induction caused an up-regulation in caspase 9, 3, 8 and 1 activity.

4.6 CURRENT NEUROPROTECTIVE STRATEGIES EMPLOYED IN THE TREATMENT AND MANAGEMENT OF PD

Parkinson’s disease is a chronic, progressive, neurological disorder. However, the cardinal symptoms appears late onset and develops throughout the course of the disease progression and responsiveness to pharmacologic therapy are highly variable (Koller, 1992; Gasser et al. 1998; Evans et al. 2005). Current therapies provide effective control of symptoms, particularly in the early stages of the disease, but disease progression is associated with the development of non-dopaminergic symptoms such as postural instability, falling, and dementia that are not adequately controlled with existing medications. Despite the advances in pharmacotherapy, the mortality rate remains largely unchanged. Existing drugs are symptomatic
and temporarily ameliorate the symptoms of PD. Pharmacotherapy for PD includes L-DOPA, dopamine agonists, monoamine oxidase (MAO) inhibitors, anti-cholinergics and most recently, Catechol-O-methyltransferase (COMT) inhibitors (Olanow and Koller, 1998; Chen and Swope 2007).

Recently, the development of a neuroprotective therapy that prevents/halts or reverses neurodegeneration is the most promising and fascinating approach for the prevention and treatment of PD. A promising treatment strategy is to provide neuroprotection to the remaining neurons to delay disease progression along with symptomatic relief and henceforth multifunctional drug therapy is an essential treatment strategy in PD management. Moreover, depending on the implicated etiological factors such as oxidative stress, mitochondrial dysfunction and deleterious inflammatory events as well as increased level of iron in SN, antioxidants, immunomodulator and iron chelators could be a possible neuroprotective therapeutic agent for the prevention and treatment of PD (Shachar et al. 2004). Therefore, neuroprotective strategies offers great promise for the prevention and treatment of PD. Currently available neuroprotective strategies are discussed herein.

4.6.1 L-dopa and dopamine agonists in PD therapy

There are several possible mechanisms by which dopamine agonists might provide neuroprotection in PD. At present, the most common treatment therapy for PD is L-3, 4-dihydroxyphenylalanine (L-dopa or levodopa), a compound that is the precursor to dopamine (DA) and still considers as a gold standard for symptomatic treatment of PD (Olanow et al. 2004). It is evident that, L-dopa is readily distributed into the central nervous system (CNS) and when taken up into dopaminergic neuron is converted to DA by the enzyme dopa-decarboxylase. Reports are available that L-dopa treatment reduced the mortality rate associated with PD (Fahn
et al. 2004), however this drug only treats the symptoms of the disease and consequently, it does not address the underlying neurodegenerative process. Chronic L-dopa treatment initially exerts side effects and induces dyskinesia (Schober, 2004) and finally becomes ineffective because the number of surviving dopaminergic neurons decreases to a point where there is no longer enough L-dopa converted to DA to compensate for the lack of endogenous nigral DA input to the striatum. Other conventional drug therapies include amantadine, an antiviral drug that has been shown to increase synaptic DA levels by inhibiting the uptake/increasing the amount of dopamine that is released as well as direct acting DA agonist such as bromocriptine, pramipexole, pergolide and ropinirole are used to treat the PD (Voelker, 2006). Further, anti-cholinergic drugs are used to counteract cholinergic input to the striatum in the absence of dopaminergic input (Schapira, 2005). The latest therapeutic interventions available as catechol-o-methyl transferase (COMPT) inhibitors such as entacapone block the breakdown of the catechol structure of DA (Kostic, 2004). In-addition to chemotherapy, deep brain stimulation and stem cell restoration treatment strategies are suggested to be more effective for treatment of motor impairment as well as other associated debilities and thus reducing the need for drug therapy. The mechanisms may involve in the dopamine agonist action are as follows:

4.6.1.1 Direct antioxidant effect

A number of in-vitro and in-vivo studies demonstrated that dopamine receptor agonists can trap a variety of free radicals, including hydroxyl radical, which is produced by the Fenton reaction. Many agonists share a hydroxylated benzyl ring structure that can scavenge free radicals (Schapira et al. 2003). In this regard, it has been demonstrated that bromocriptine, a dopamine receptor agonist, can scavenge hydroxyl radicals in both in-vitro and in-vivo conditions (Kondo et al.1994; Muralikrishnan et al. 1998). Another drug apomorphine can also scavenge free radicals as demonstrated in a study with isolated rat brain mitochondria (Gassen et al.1996). Similarly,
pergolide and pramipexole have also been reported to scavenge hydroxyl, superoxide and nitric oxide radicals in cell line cultures (Gomez-Vargas et al. 1998; Nishibayashi, 1996). In addition, reports are available that indicate the ability of ropinirole to increase the concentrations of glutathione, catalase and superoxide dismutase (Iida et al. 1999). It is reported that the heterocyclic rings in pramipexole, ropinirole, quinpirole might be responsible for their free radical scavenging effects. Disadvantage of this approach is that the amount of drug needed to impart antioxidant effect is quite high, often in micromolar range, which is not achievable during routine use of these drugs in PD patients to alleviate symptoms. Therefore, it is ambiguous whether the direct antioxidant properties of dopamine receptor agonists can cause neuroprotection or not (Schapira et al. 2003).

4.6.1.2 Monoamine oxidase-B (MAO-B) inhibitors

Dopamine is metabolized by monoamine oxidase-B (MAO-B) to form 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide. Use of MAO-B inhibitors, such as selegiline (deprenyl) and rasagiline, increases the concentration of available dopamine while reducing the oxidative stress that contributes to the progression of the disease by reducing the hydrogen peroxide that results from the reaction (Magyar and Szende, 2004; Mandel et al. 2005; Yamada and Yasuhara, 2004). Since treatment for PD necessarily follows disease diagnosis, and thus dopamine levels are already diminished, MAO-B inhibitors provide mild therapeutic effect for managing PD symptoms (Singh et al. 2007). Though, MAO-B inhibitors are typically used in combination with L-DOPA administration, despite the severe side effects (Rinne et al. 1991).
Table 4.1 Summary of etiological factors, mechanisms and protective strategies used for PD therapy

<table>
<thead>
<tr>
<th>Factors</th>
<th>Pathogenesis of PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
<td>Genetic</td>
</tr>
<tr>
<td></td>
<td>Environmental</td>
</tr>
<tr>
<td></td>
<td>Genetic susceptibility to environmental factors</td>
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<tr>
<td>Mechanism in pathogenesis</td>
<td>Inflammation</td>
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<td></td>
<td>Mitochondrial dysfunction</td>
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<td></td>
<td>Oxidative and nitrosative stress</td>
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<td></td>
<td>Protein aggregation and misfolding</td>
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<tr>
<td></td>
<td>Excitotoxicity</td>
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<tr>
<td></td>
<td>Loss of neurotrophic factors</td>
</tr>
<tr>
<td>Mechanisms of dopaminergic cell death</td>
<td>Loss of mitochondrial membrane potential</td>
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<tr>
<td></td>
<td>Mitochondrial permeability transition</td>
</tr>
<tr>
<td></td>
<td>Loss of cytochrome-c from mitochondria</td>
</tr>
<tr>
<td></td>
<td>Accumulation of cytosolic calcium</td>
</tr>
<tr>
<td></td>
<td>Activation of apoptotic pathways</td>
</tr>
<tr>
<td>Therapeutic approaches and potential targets</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial (coenzyme Q10, Carnitine, uncoupling protein 2 expression)</td>
</tr>
<tr>
<td></td>
<td>Excitotoxicity (antiglutamate)</td>
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<tr>
<td></td>
<td>Calcium overload (calcium channel blockers)</td>
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<tr>
<td></td>
<td>Protein accumulation (heat shock proteins, proteasomal enhancers)</td>
</tr>
<tr>
<td></td>
<td>Antiapoptotic stabilizers</td>
</tr>
</tbody>
</table>

4.7 EXPERIMENTAL MODELS EMPLOYED IN PD STUDIES

Animal models are valuable to the extent to which they accurately simulate the pathogenic, histological, biochemical or clinical features of neurodegenerative disorders including PD. Animal models of PD also offer the possibility to test and develop rational therapeutic strategies that may benefit patients with this progressive neurodegenerative disease. It is desirable that an ideal animal model should reproduce the progressive, selective nigrostriatal dopaminergic neurodegeneration and recapitulate most of the cardinal features of PD. In-addition to the loss of nigrostriatal dopamine neurons; a key determinant that differentiates PD from other neurodegenerative disorders is the LBs inclusion formation, which should be reproduced in the
models. However, there is no perfect animal model of PD that is able to recapitulate every aspect of the disorder. Though, currently available models can reproduce specific features of the disease. Moreover, the main purpose of the selection of an appropriate animal model of PD will be based on ability to produce reliable, reproducible lesions of the NSDA (nigro-striatal dopamine) pathway that are in keeping with the severity of NSDA loss observed in PD. Furthermore, neurotoxin-based models of PD have a long history and represent the most important characteristic symptoms of PD, while genetic models have failed to recapitulate the key neurobehavioral or pathological features of PD (Cannon and Greenamyre, 2010). Extensive study of these models has defined important etiological factors of dopaminergic cell death including oxidative stress, mitochondrial dysfunction, excitotoxicity, neuro-inflammation and nitric oxide which are presumably critical for the nigral degeneration (Blum et al. 2001; Singh and Dikshit, 2007). Furthermore, the neurotoxin induced models can serve as valuable tools for the assessment of efficacy and side-effects of symptomatic treatments of PD and has offered a basis for the development of novel therapeutic strategies (Tetrud and Langston, 1989; Bloem et al. 1990; Blanchet et al. 1998; Fox and Brotchie, 2010). A number of neurotoxin-based animal models of PD have been developed that produce specific NSDA neurons loss. The most commonly neurotoxins for this purpose are 6-hydroxydopamine (6-OHDA), N, N-dimethyl-4,4-bipiridinium (paraquat), rotenone and 1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine (MPTP). Paraquat, rotenone and MPTP were identified as herbicide/pesticides risk factors for PD in humans and subsequently developed for use in animals to recapitulate the disease in the laboratory. Brief description about the common toxin-induced animal models employed for neuroprotective studies has been mentioned herein.

4.7.1 Six-hydroxy dopamine (6-OHDA) model

It was the first chemical agent discovered that possesses specific neurotoxic effect on catecholaminergic pathways (Ungerstedt, 1971; Sachs and Jonsson, 1975). 6-OHDA is a
hydroxylated analogue of dopamine and thus uses the same catecholamine transport system and produces specific degeneration of catecholaminergic neurons. Several reports are available indicating that systemic 6-OHDA administration is not able to cross the blood–brain barrier. Therefore, 6-OHDA can be stereotaxically injected into SNpc or the ascending medial fore brain (MFB) or the striatum for the specific target to nigrostriatal dopaminergic pathway and model development. It is evident that following 6-OHDA injection, dopaminergic neurons start degenerating within 24 hours and takes 2-4 weeks to induce striatal dopamine depletion and the magnitude of the lesion is dependent on the amount of 6-OHDA injected and the site of injection (Deumens et al. 2002). Injection of 6-OHDA directly to the striatum causes a retrograde degeneration of the nigrostriatal system over a period of weeks and has been used to mimic the slow progressive nature of PD (Deumens et al. 2002; Przedborski et al. 1995). Furthermore, unilateral 6-OHDA injection leads to asymmetric rotational motor behaviour after administration of dopaminergic drugs like amphetamine or apomorphine, due to physiologic imbalance between the lesioned and the unlesioned striatum. This can be quantified and correlated with degree of neuronal lesion (Ungerstedt, 1971). The major disadvantage of the 6-OHDA model is that it differs from progressive degeneration of the dopaminergic nigral neurons in PD. The 6-OHDA model lesion has been used to ascertain the efficacy of antiparkinsonian compounds (Schwarting and Huston, 1996; Saleem et al. 2005; Khuwaja et al. 2011).

4.7.2 Paraquat model

It is a herbicide that is naturally found in the environment and is associated with increased risk for PD (Liou et al. 1997; Shimizu et al. 2003; Dinis-Oliveira et al. 2006). Paraquat enters DA neurons via the DAT (Shimizu et al. 2003) however; non-DAT mediated transport may also occur (Richardson et al. 2005). Once inside the neurons paraquat reacts with nicotinamide adenine dinucleotide (NADPH, NADH) in a reaction catalysed by diaphorases to form reactive
Diaphorases are enzymes that transfer electron from electron donors (NADH, NADPH) to other molecules (Liochev et al. 1994; Shimada et al. 1998). The reactive paraquat form in the first reaction can then react with molecular oxygen to form superoxide radicals (Day et al. 1999; Przedborski and Ischiropoulos, 2005). Manning-Bog et al (2002) reported that paraquat induced superoxide generation leads to oxidative stress, the destruction of NSDA neurons, and the formation of neuronal inclusions in the frontal cortex reminiscent of LBs. However, the degeneration of cortical neurons has not been reported. The advantage of the paraquat model is that it causes the formation of protein inclusions in neurons similar to LBs, allowing the molecular mechanisms that result in the formation of LBs to be investigated. The disadvantage of this model is that NSDA cell loss has not been consistently reported and it is not known if NSDA neurons are particularly vulnerable to paraquat toxicity (Thiruchelvam et al. 2000).

4.7.3 Rotenone model

This model of PD is based on chronic systemic exposure of rats to rotenone, a pesticide and complex I inhibitor (Betarbet et al. 2000). Rotenone is a lipophilic compound that easily crosses the blood brain barrier. The rotenone model appears to be an accurate model, since chronic exposure to rotenone resulted in uniform and selective dopaminergic neuronal damage, selective striatal oxidative damage, and formation of ubiquitin and α-synuclein positive inclusions in nigral cells (Greenamyre et al. 2003; Saravanan et al. 2006). The major problem associated with this animal model is its nature of variability, with only some animals showing lesions.

4.7.4 MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) model

The emergence of neurotoxin 1-methyl 4-phenyl 1,2,3,6 tetrahydropyridine (MPTP) as an experimental tool to produce a reliable model of PD has provided new insight into the etiology
and pathogenesis of idiopathic PD (Dauer and Przedborski 2003; Kurosaki et al. 2004; Fox and Brotchie 2010). MPTP is highly lipophilic, and thus readily crosses the blood-brain barrier (BBB). After it enters the CNS, monoamine oxidase B (MAO-B) expressed in astrocytes converts MPTP to its toxic by-product, 1-methyl-4-phenylpyridinium (MPP⁺), which is then released by astrocytes and is selectively taken up by dopaminergic neurons via the dopamine transporter (Dauer and Przedborski 2003; Ransom et al. 1987). Once accumulated in dopaminergic neurons, MPP⁺ concentrates in mitochondria, where it interferes with mitochondrial complex I of the electron transport chain (ETC). This interference reduces ATP availability and increases the relative concentrations of reactive oxygen species (ROS), leading to apoptosis in dopaminergic neurons (Kitamura et al. 2000; Kitamura et al. 2003; Speciale, 2002). Due to its ability to cross the BBB, MPTP is utilized for in-vivo experiments, while the less hazardous MPP⁺, which does not cross the BBB, is used primarily for in-vitro experiments to reduce potential exposure to experimenters. Pathological feature of the MPTP-induced model of PD most closely resemble PD pathological hallmarks (Fox and Brotchie 2010; Shimohama et al. 2003) especially in primates, where severe and irreversible Parkinsonian features (nigral dopaminergic neuron degeneration and appearance of cytoplasmic inclusion bodies) are observed. One major departure from the sporadic PD observed in humans is the acute onset following MPTP treatment, which makes it a desirable experimental model for PD research. However, chronic PD symptoms can be induced by chronic administration of lower-doses of MPTP (Kopin and Markey 1988; Schintu et al. 2009). The C57BL/6 mouse strain is more susceptible to MPTP toxicity, so that commonly used in laboratory experiments (Przedborski and Jackson-Lewis 1998; Sedelis et al. 2000; Filipov et al. 2009). Despite the minor differences, the MPTP model is the gold-standard among PD models because in humans and non-human primates it produces a Parkinsonian syndrome characterized by all the features of PD: tremor, rigidity, slowness of movement, postural instability, and
freezing (Dauer and Przedborski, 2003). In-addition, similar to PD patients, increased susceptibility to MPTP is observed as they aged (Irwin et al. 1993; Ovadia et al. 1995). Furthermore, response to L-DOPA treatment and development of motor complications as a result of chronic therapy are similar to those observed in PD patients (Dauer and Przedborski 2003). Moreover, MPTP intoxication results in nigrostriatal dopaminergic pathway with 50% to 93% cell loss in the substantia nigra pars compacta and more than 99% loss of dopamine in the striatum (Hantraye et al. 1993). Rats are resistance to MPTP toxicity and mouse strains vary widely for sensitivity to the toxin. Neurochemical changes following MPTP exposure include decreased level of dopamine and its metabolites in the striatum, increased oxidative damage as evidenced by increased lipid peroxidation, increased 3-nitrotyrosine levels and diminished concentration of antioxidants, such as glutathione (GSH) and superoxide dismutase (SOD).

Figure 4.5: A schematic representation of the mechanisms/cascades involved in toxicity of MPTP (Gerlach and Riederer 1996).
The systemic MPTP administration in mice causes an irreversible loss of NSDA neurons and the development of motor deficits that resemble PD symptoms (Jackson-Lewis et al. 1995; Sedelis et al. 2001). NSDA neurons are more vulnerable to the toxic effects of MPTP, compared to the norepinephrine neurons and even other DA neurons that express the DAT (Chiueh et al. 1984). The effect of MPTP administration on the time course, extent and mechanism of cell death is highly dependent on the dosing regimen. In general, acute administration paradigms where high doses of the neurotoxin are administered over a brief period (usually a cumulative dose of 80 mg/kg in one day) results in very rapid, irreversible and severe NSDA cell loss that is primarily necrotic (Bezard et al. 1997; Smeyne and Jackson-Lewis, 2005). However, the rapidity and severity of cell loss in the acute treatment model does not match the slowly progressive nature of early PD where apoptotic cell death is known to occur (Mochizuki et al. 1996; Anglade et al. 1997). Sub-chronic dosing regimens (25 mg/kg per day for five consecutive days) and prolonged-chronic regimens (20 mg/kg, twice a week for five consecutive weeks) have been developed that distribute the total dose of MPTP over several days, causing a prolonged impairment of mitochondrial function and chronic exposure to ROS. Using these dosing regimens cell death occurs from both apoptotic and necrotic mechanisms, which more accurately reflects cell death in PD (Bezard et al. 1997; Tatton and Kish, 1997; Novikova et al. 2006). Moreover, despite a higher cumulative dose of MPTP the lesion produced by the neurotoxin is less severe than in the acute model and more reminiscent of the extent of NSDA neurons loss observed in early PD. Limitations of MPTP model are its failure to mimic progressive nature of PD as well as does not produce LBs in rodents (Przedborski et al. 2001).
Overall impact of MPTP cytotoxicity and neuroprotective strategies employed in the present study

**Neurochemical alterations**
- Dopamine and its metabolite
- Serotonin and nor-adrenaline

**Behavioral alterations**
- Motor coordination
- Gross behavioral activity
- Grip strength and depression

**Biochemical alterations**
- Mitochondrial Complex activity
- Endogenous AO, LPO and LH
- Protein carbonyls
- NO and nNOS
- TH and MAO-B activity

**Inflammatory markers**
- Inflammatory cytokines
- GFAP and iNOS

![Fig 4.6: Schematic diagram of MPTP induced neurotoxicity and behavioral, biochemical and neurochemical alterations and hypothesized neuroprotection exerted by withanolides and curcuminoids.](image)

### 4.7.5 Genetic model

A number of genetic mutations are associated with familial PD; whereas familial PD only accounts for less than 10% of all reported cases (Gasser 2001; Lim and Chee-Hoe Ng 2009). The identification of the genes that are associated with PD pathogenesis has been instrumental to understand the neurodegenerative process that occurs in the disease (Dawson et al. 2010). In particular, mutation, duplications or triplications in the genes encoding the α-synuclein protein
are reported to results in heritable PD and cause degeneration of NSDA neurons (Polymeropoulos et al. 1997; Kruger et al. 1998; Singleton et al. 2003). This model system focused on the use of transgenic mice or drosophila, which express the wild type or mutated α-synuclein. Transgenic mice over expressing human α-synuclein demonstrated a number of features of PD, including loss of nigrostriatal dopaminergic nerve terminal in striatum, development of α-synuclein and ubiquilin-positive cytoplasmic inclusion, and motor impairments (Masliah et al. 2000).

After comprehensive literature survey, it is apparent that more than one etiological factor contributes to the pathogenesis of idiopathic PD. These factors may include increased genetic susceptibility and exposure to environmental factors. However, the existing animal model will undoubtedly continue to advance our understanding of the pathophysiology of PD. Such models of PD also offer the possibility to test and develop rational therapeutic strategies that will hopefully benefit patients with this progressive neurodegenerative disease. Considering these points in view, the present experimental study has been designed. To develop reliable PD model, acute dose regimen of MPTP (20x4/kg; one day) was selected and subsequently intra-peritoneally (i.p.) injected to the C57Bl/6 mouse strain.

4.8 PROMISING MEDICINAL PLANTS OF INDIAN SYSTEM OF MEDICINE (ISM) USED IN PD THERAPY

In the Indian system of medicine (ISM) prominently used by folklore, nature has been the source of treatment for various disorders from time immemorial; plant based therapeutic drug candidates continue to play an essential role in the primary health care of 80% of the world’s underdeveloped and developing countries (Valianthan, 1998). Moreover, due to long history of use, perceived effectiveness, minimal side effects and relatively low cost, herbal drugs are widely prescribed even when their mode of action, targets and lead compounds are unknown (Pickup and Williams, 1991). Since alternative system of medicine possess immense potential in disease management, World Health Organization (WHO) has also recommended and encouraged
this practice especially in countries where access to the conventional treatment of chronic disorders are not adequate (WHO 1980). In this context, medicinal plants have always been major source of putative drug candidates and are extensively used in the Indian System of Medicine (ISM) including Ayurveda and Ayurvedic practitioner used to treat various chronic disorders on their own experience-based theories. In Ayurveda, PD is defined as “Kampavata” (Manyam, 1990) and various prescriptions/formulations have been recommended for the treatment and management of PD. Ayurvedic prescriptions based on an alternative medicine source suggest that, a concoction in cow’s milk with powdered mixture of *Mucuna pruriens* and *Hyoscyamus reticulates* seeds with *Withania somnifera* and *Sida cordifolia* roots is recommended for the treatment and management of PD. Furthermore, clinical study conducted by Rabey et al., (1993) showed that ingestion of *Vicia faba* seeds produced an increase in the levodopa plasma levels, which correlates with motor performance improvements. Several other reports are also available that a *Vicia faba* bean contains high phenolic content with antioxidant properties (Shetty et al. 2002). Likewise, the seeds of *Mucuna pruriens* (Damodaran and Ramaswamy, 1937) and *Vicia faba* (Lattanzio et al. 1982) were used on PD treatment for their levodopa content while the extracts of Datura’s seeds were also used due to the anticholinergic effects (Hussain and Manyam, 1997). Cumulative research findings has suggested that various phytoconstituents are available that act as monoamine oxidase inhibitors, such as *Banisteria capi* (Sanchez-Ramos, 1991) and *Nicotiana tabacum* (Norman et al. 1982). Moreover, recent findings suggested that other plants with anti-parkinsonian activity studied on animal models including *Centella asiatica*, *Quercus infectoria*, *Nardostachys jatamansi*, *Hypericum perforatum*, *Ginkgo biloba*, green tea and curcuminoids (Table, 4.2). Ramaswamy et al (1970) has reported that the ethanolic extract of *Centella asiatica* did not inhibit the tremor induced by tremorine in mice in a dose of 50 mg/kg, i.p., however, the methanolic extract of *Quercus infectoria* showed a weak activity in mice at a dose of 500 mg/kg i.p., in the similar experiment (Dar et al. 1977). Bastianetto et al. (2002) has demonstrated that *Ginkgo biloba* leaves extract rich in flavonoids, protected hippocampal neurons
from neurotoxicity induced by nitric oxide or beta-amyloid-derived peptide. The acetone extract of *Ginkgo biloba* leaves showed protective effects, in rats with a dose of 50 mg/kg, i.p, against the PD induced by 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (Yang et al. 2001). Moreover, the green tea extract and (-)-epigallocatechin-3-gallate showed neuroprotective effects in the same 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine model of PD in mice. In-addition, tea extract have been previously reported to possess potent antioxidant-radical scavenging activities. Green tea and black tea extracts attenuated the neuronal apoptosis induced by 6-hydroxydopamine. This neuroprotection was attributed to the antioxidant actions of the polyphenolic constituents of tea extracts that have good capability to penetrate the blood-brain barrier (Levites et al. 2002). Neuroprotection may also be obtained with cannabinoids, experiments suggested that these compounds rescued dying neurons in experimental acute neuronal injury, such as cerebral ischemia and traumatic brain injury and provided symptomatic relief in experimental models of chronic neurodegenerative diseases, such as multiple sclerosis and Huntington’s disease (Grundy, 2002). The central cannabinoid receptors are densely located in the output nuclei of the basal ganglia (globus pallidus, substantia nigra pars reticulata), suggesting involvement in the motor activity regulation. There are a limited number of clinical trials in humans demonstrating that cannabinoids might be useful in the treatment of movement disorders, with evidence of reduction of levodopa- induced dyskinesia in PD and some forms of tremor and dystonia (Muller-Vahl et al. 1999). Among all, *Mucuna pruriens* seed preparations are in contemporary use for the treatment of PD in India (Manyam and Sanchez-Ramos, 1999). It is evident that seed of *Mucuna pruriens* contains levodopa, which ameliorates the parkinsonism associated symptoms including dyskinesia in 6-OHDA lesioned rat model of PD (Lieu et al. 2010) and possess better anti-Parkinsonian activity compared with levodopa (Manyam et al. 2004). Moreover, clinical trials showed positive effects of seed powder formulation of *Mucuna pruriens* on PD patients with rapid onset of action and longer on time but without concomitant increase in dyskinesias (Katzenschlager et al. 2004). Commercial preparations of *Mucuna pruriens* (HP-200, Zandopa)
are now available for the treatment of PD. Recently, Maheswari et al (2010) has demonstrated that, oral administration of mucuna seed powder and withania root powder has synergistically attenuated the MPTP-induced toxicity in mice brain. Considering the scientific literature and evidences, it is noteworthy, that medicinal plants possess immense potential as a therapeutic candidate for the prevention and treatment of neurodegenerative disorders including PD. Henceforth, it is of urgent need to investigate the mechanisms behind their protective efficacy, to validate their efficacy profile for their wide acceptance as well as for the drug development to treat PD.

**Table 4.2: Summary of pharmacological studies conducted on various extracts and active phytocomponents used in experimental studies of PD**

<table>
<thead>
<tr>
<th>Medicinal Plant</th>
<th>Extract/compound</th>
<th>Model</th>
<th>Pharmacological effect and targets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthopanax senticosus (Harms)</td>
<td>Sesamin Whole extract</td>
<td>Rotenone and MPTP (Rat)</td>
<td>Preventing loss of DA cells, bradykinesia, depression</td>
<td>Fujikawa et al. (2005a,b)</td>
</tr>
<tr>
<td>Allium sativum L.</td>
<td>S-allylcysteine</td>
<td>MPTP (Mice)</td>
<td>Preventing loss of DA cells, antioxidant</td>
<td>Rojas et al. (2010)</td>
</tr>
<tr>
<td>Bacopa monnieri (L.)</td>
<td>Standardized whole plant powder</td>
<td>Rotenone (Drosophila)</td>
<td>Antioxidant, inhibiting DA depletion</td>
<td>Hosamani and Muralidhara (2009)</td>
</tr>
<tr>
<td>Centella asiatica (L.)</td>
<td>ethanol extract</td>
<td>MPTP Rats</td>
<td>Antioxidant</td>
<td>Haleagrahara and Ponnusamy (2010)</td>
</tr>
<tr>
<td>Plant/Extract</td>
<td>Effect/Model</td>
<td>Chemical</td>
<td>Properties</td>
<td>References</td>
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<tr>
<td></td>
<td>MPTP (Mice)</td>
<td>MAO-B inhibition</td>
<td>Khuwaja et al. (2011) Ojha et al. (2012)</td>
<td></td>
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<tr>
<td></td>
<td>MPTP (Mice)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Morus alba L. Ethanol extract</td>
<td>MPP+ SH-SHYSY5Y and MPTP (Mice)</td>
<td>Preventing loss of DA cells, bradykinesia</td>
<td>Kim et al. (2010b) Chao et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Oxyresveratrol</td>
<td>6- OHDA SHSY5Y</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>SHSY5Y</td>
<td>Antioxidant, anti-apoptosis, ↓JNK, ↑Akt, ↑SIRT1</td>
<td></td>
<td></td>
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<tr>
<td>Nardostachys jatamansi Root extract</td>
<td>6-OHDA</td>
<td>Neuroprotective</td>
<td>Ahmad et al. (2006)</td>
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<tr>
<td></td>
<td>6-OHDA (Rat)</td>
<td>Antioxidant</td>
<td>Ahmad et al. (2005a)</td>
<td></td>
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<tr>
<td>Zingiber officinale Rosc. Eugenol</td>
<td>6-OHDA (Rat)</td>
<td>Antioxidant and DA inhibition</td>
<td>Kabuto and Yamanushi (2011)</td>
<td></td>
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<tr>
<td></td>
<td>Zingerone</td>
<td></td>
<td>Kabuto et al. (2005)</td>
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</table>
4.9 RATIONALE BEHIND THE SELECTION OF WITHANOLIDES AND CURCUMINOIDS

Cumulative research findings suggested that *Withania somnifera* (*L.*) and *Curcuma longa* (*L.*) possess immense therapeutic potential and have shown neuroprotective effect through its anti-oxidant, anti-inflammatory and immunomodulatory activity. Among various phytoconstituents, withanolides (steroidal lactones/alkaloids) and curcuminoids (polyphenolic) fractions are believed to be the active principal constituents to attribute their neuroprotective activity. As discussed in the present chapter under etiological factors of PD section, it is striking that anti-oxidants and anti-inflammatory agents have been shown to be effective on PD treatment (Prasad et al. 1999; Miquel et al. 2002; Choi et al. 2002; Gao et al. 2003c; Zbarsky et al. 2005; McGeer and McGeer 2007). In this context, neuroprotective strategies through antioxidant and immunomodulation have great prospects in PD management. Pertaining to it, reports are available to corroborate that *Withania somnifera* possesses adaptogenic, nootropic and anxiolytic properties and the major constituent withanolides correlated to this effect (Singh et al. 2003; Bhatnagar et al. 2009). Moreover, Kuboyama et al (2005) reported that root extract of WS attributed neuroprotective activity through synapse reconstitution. Similarly, curcumin the major constituent of curcuminoids possess broad spectrum of pharmacological activities including radical scavenger, chelation and anti-inflammatory properties (Motterlini et al. 2000; Chainani-Wu 2003; Chattopadhyay et al. 2004; Epstein et al. 2010). Khopde et al (1999) reported that curcumin possess ten times better antioxidant activity than vitamin E and it may be the reason for its protective effect over various oxidative insult and injuries (Song et al. 2001; Iqbal et al. 2003; Thiagarajan and Sharma 2004; Somparn et al. 2007). Moreover, curcumin has been reported to act as a scavenger of oxygen free radicals and to activate endogenous antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase (Ruby et al. 1995; Reddy and Lokesh 1994). Curcumin has also been reported to act as an electrophile (Kluth et al. 2007; Dinkova-Kostova and Talalay 1999) and it has been shown to induce the activities of
phase II detoxification enzymes (Dinkova-Kostova and Talalay 1999), thus significantly inhibits
 generation of reactive oxygen species (ROS), like super oxide anions, hydrogen peroxide and
 nitrate radicals, by activated macrophages (Joe and Lokesh 1994). Consequently, the
 neuroprotective effects of curcumin relevant to PD are likely to be associated with its antioxidant,
 anti-inflammatory and chelating properties (Zbarsky et al. 2005; Chen and Le 2006; Jagatha et al.
 2008). Taken together, it can be assume that due to the presence of potent phytoconstituents,
 withanolides and curcuminoids have great potential as neuroprotective agent for the prevention
 and treatment of PD.

4.9.1 Brief record of medicinal use, phytochemistry, pharmacology and antiparkinsonian
activity of the *Withania somnifera* and its active phyto-constituents

*Withania somnifera* (WS) is a small, erect, evergreen woody under shrub reaches about
30-150cm in height; belongs to solanaceae family. WS is known by various vernacular names as
in Hindi it is known as asgandh; English, winter cherry; turangi, gandha; in Bengali, aswagandha;
in Gujarati, ghodakun and ghoda; in Telgu, vajigandha and pulivendram, in Tamil, amukkira; in
Malyalam, amukkuram and in Kannada, viremaddinagaddi.

*Withania somnifera* popularly known as aswagandha/Indian Ginseng is a reputed Indian
medicinal plant with a broad spectrum of therapeutic potential and used for over 4000 years in
Indian System of Medicine (ISM). Ashwagandha (trade name of *Withania somnifera*) has been
used by all age groups and both sexes and even during pregnancy without any toxic effects
(Mishra et al. 2000). Traditionally, the plant has been used as an antioxidant, adaptogen,
aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers,
bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support
the use of WS for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia
and PD. Recently, WS has been used to inhibit the development of tolerance and dependence on
chronic use of various psychotropic drugs. Moreover, this plant has been studied extensively for
its biologically active constituents and has yielded several steroidal lactones called withanolide (Jayaprakasam and Nair 2003; Matsuda et al. 2001). In-addition, various pharmacologic studies indicated that active phytoconstituents as withanolide, withaferin A, inhibits angiogenesis, metastasis and quinone reductase activity (Mohan et al. 2004; Leyon and Kuttan 2004; Misico et al. 2002). Some of them have been shown to preferentially affect in the cholinergic signal transduction cascade of the cortical and basal forebrain, indicating their promise for the treatment of Alzheimer’s disease (Kuboyama et al. 2005). Though WS/withanolides possesses broad spectrum of pharmacologic activity but still lots of research is required to answer its mode action on different targets to validate neuroprotective activity. In this context, present study is an effort to investigate the neuroprotective effect of withanolides in the MPTP-induced PD model.

![Withania somnifera (L.) aerial part Dried root](image)

### Figure 4.7: Withania somnifera (L.) aerial part Dried root

#### 4.9.2 Pre-clinical and clinical studies on *Withania somnifera* and its principal phytoconstituents

Although a lot of pharmacological investigations have been carried out on the phytoconstituents of WS, still there is lot of scope and need to be explored the broad spectrum of medicinal use of its active phytoconstituents and their mode of action. Summary of the various studies are discussed herein.
4.9.3 Phytochemistry of *Withania somnifera* and its principal components

The phytoconstituents of WS are chemically very complex, even though more than 80 compounds are characterized so far. The biologically active phyto constituents are alkaloids-ashwagandhine, cuscohygrine, anahygrine, tropine, steroidal compounds, including ergostane type steroidal lactones, withaferin A, withanolides A-y, withasomniferin-A, withasomniferin, withasomniferols A-C, withasomniferin A, withasomniferol, withasomniferols A-C, and withanone. The major constituents of withania roots are the steroidal alkaloids and steroidal lactones; collectively constitutes withanolides (Elsakka et al. 1990; Mishra et al. 2000). Among all, withaferin A, termed as phytosteroid, major bioactive constituent of withanolides with multiple pharmacological activity (Lavi et al. 1965; Kuboyama et al. 2005). In addition, other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitoindoside ix and x) (Elsakka et al. 1990; Ganzera et al. 2003). Along with these phyto constituents, it also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of amino acid including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron.

The reported alkaloids present in WS are anaferine [bis (2-piperidylmethyl) ketone]; isopelletierine; tropine; pseudotropine; 3α-tigloyloxtropine; 3-tropyltigloate; cuscohygrine; dl-isopelletierine; anahygrine; hygrine; mesoanaferine; choline; somniferine; withanine; withananine; hentriacontane; visamine; pseudowithanine and ashwagandhine. Withaniol (mixture of withanolides) and number of withanolides including withaferine-A; withanolide N and O; withanolide D; withanolide p and 8; withanolide Q and R; withanolide y, 14α- hydroxyl steroids and withanolides G, H, I, J, K and U (Kirson and Glotter 1980). Seven new withanolide glycosides called withanosides I, II, III, IV, V, VI and VII had been isolated and identified (Matsuda et al. 2001). Among these constituents, withaferin A and withanolide D has been
attributed maximum pharmacological activity in different studies. Henceforth, taken together, it can be assumed that, various active phytoconstituents attributes its potent pharmacological activity.

![Figure 4.8: The basic skeleton of withanolides](image)

**4.9.4 Chemistry of withanolides**

The withanolide skeleton may be defined as a 22-hydroxyergostan-26-oic acid-26, 22-lactone. There are many novel structural variants of withanolides with modifications either of the carbocyclic skeleton or the side chain and these have often been described as modified withanolides or ergostan type steroids related to withanolides. These compounds are generally polyoxygenated and it is believed that plants elaborating them possess an enzyme system capable of oxidizing all carbon atoms in a steroid nucleus. The characteristic feature of withanolides and ergosane-type steroids is one C8 or C9-side chain with a lactone or lactol ring but the lactone ring may be either six-membered or five membered and may be fused with the carbocyclic part of the molecule through a carbon-carbon bond or through an oxygen bridge. Appropriate oxygen substituents may lead to bond scission, formation of new bonds, aromatization of rings and many other kinds of rearrangements resulting in compounds with novel structures (Tursunova et al. 1977; Glotter, 1991; Kirson et al. 1971).
Withaferin A R1=OH, R2=H, R3=H; Withanolide D R1=H, R2=OH, R3=H; 27-Deoxywithaferin A R1=H, R2=H, R3=H; 14α-OH, R1=H, R2=H; 17α-OH, R1=H, R2=H, R3=H; 27-Hydroxywithanolide D R1=OH, R2=OH, R3=H; 14α-OH, R1=OH, R2=H, R3=H; 17α-OH, R1=OH, R2=H, R3=H; Di-hydro deoxy withaferin A 2,3-diH, R1=H, R2=H, R3=H; Di-hydro withaferin A 2,3-diH, R1=H, R2=OH, R3=H; 17-Hydroxywithaferin A, R1= R3=OH, R2=H.

4.9.5 Pharmacological activity of Withania somnifera and its phytoconstituents

4.9.5.1 Antioxidant effect

Bhattacharya and co-workers (2001) have reported that, the active principle constituents of WS, sitoindosides VII-X and withaferin A demonstrated promising antioxidant activity through modulating free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the frontal cortex and striatum of the rat brain. In another study Dhuley et al (1998) has reported that administration of aqueous suspension of WS root extract attenuated lipid peroxidation level in lipopolysaccharide (LPS)-induced model.

4.9.5.2 Nootropic effect

The study conducted by Dhuley (2001) has reported that root extract of WS dose
dependently exhibited nootropic effect in naive and amnesic mice. In another study Schliebs and co-workers (1997) have demonstrated that sitoindosides VII-X and withaferin isolated from roots of WS exerted significant effect on brain cholinergic, glutamatergic and GABAergic receptors in rats. They have postulated that these changes were accompanied by enhanced M1-muscarinic-cholinergic receptor binding in lateral and medial septum as well as in frontal cortices, whereas the M2- muscarinic receptor-binding sites were increased in a number of cortical regions including cingulate, frontal, parietal, and retrosplenial cortex. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition enhancing and memory-improving effects of WS extracts in animals and in humans. Recently, Kuboyama and co-workers (2006) have reported that oral administration of withanoside IV significantly improved memory deficits in Abeta-injected mice and prevented loss of axons, dendrites, and synapses. Further they have suggested sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV. They postulated that sominone induced axonal and dendritic regeneration and synaptic reconstruction significantly in cultured rat cortical neurons damaged by Abeta. Withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease and that the active principle after metabolism is sominone. Naidu and co-workers (2006) have reported that chronic WS administration significantly reversed reserpine-induced retention deficits. In different study with WS root extract improved retention of a passive avoidance task in a step-down paradigm in mice. WS also reversed the scopolamine-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS).

4.9.5.3 Antiparkinsonian properties

Antiparkinsonian effects of WS root extract have been reported due to potent antioxidant, anti-peroxidative and free radical quenching properties in diseased conditions. (Ahmad et al. 2003). In a recent study Kumar et al (2006) has reported that, root extract of WS significantly
inhibited haloperidol or reserpine-induced catalepsy and provide hope for treatment of PD. Several recent studies has suggested that WS significantly reversed the catalepsy, tardive dyskinesia and 6-hydroxydopamine elicited toxic manifestations and may offer a new therapeutic approach to the treatment of PD (Saleem et al. 2005). WS root extract improves catecholamines and physiological abnormalities seen in a MPTP-induced mouse model of PD (RajaSankar et al. 2009)

4.9.5.4 Anti-inflammatory properties

The anti-inflammatory effects of WS have been studied in several studies. Anbalagan and his co-workers (1981) has reported that WS root extract (1 g/kg, oral) reduced Freund’s complete adjuvant induced inflammation in rats. Anbalagan et al (1984) have reported that WS caused dose-dependent suppression of 2-macroglobulin in the serum of rats inflamed by sub-plantar injection of carrageenan suspension. Begum et al.1987 reported that WS root powder decreased air pouch granuloma induced by carrageenan on the dorsum of rats. WS decreased the glycosaminoglycans content in the granuloma tissue more than hydrocortisone treatment. WS also uncoupled the oxidative phosphorylation by significantly reducing the ADP/O ratio in mitochondria of granuloma tissue. In other studies, WS root extract showed significant reduction in both paw swelling and bony degenerative changes in Freund’s adjuvant-induced arthritis in rats as observed by radiological examination (Begum et al. 1988). Few recent studies have been conducted to investigate the mechanism of action for the anti-inflammatory properties of WS (Rasool et al. 2006). Recent studies also suggest that WS exerts anti-inflammatory activity that is may be due to its mechanism of action on cyclooxygenase inhibition. The role of WS as immunomodulator has been extensively studied. WS root extract also enhance total white blood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group (Davis et al. 2002). Recent research study suggests a possible mechanism behind the increased cytotoxic effect of
macrophages exposed to WS extracts. Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganisms and tumor cells. Iuvone et al. (2003) demonstrated that root extract of WS increased NO production in mouse macrophages in a concentration-dependent manner. This effect was attributed to increased production of inducible nitric oxide synthase, an enzyme generated in response to inflammatory mediators and known to inhibit the growth of many pathogens. In another study, Glyco withanolides and a mixture of sitoindosides IX and X isolated from WS, both produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis, and increased activity of the lysosomal enzymes. Root extract of WS was tested for immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone (Ziauddin et al. 1997). Significant increases in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in WS-treated mice compared to untreated control mice. WS root extract showed potent inhibitory activity towards the complement system, mitogen induced lymphocyte proliferation and delayed-type hypersensitivity reaction (Rasool et al. 2006).
Table 4.3: Summary of Pharmacological activities of standard root extract and active phytoconstituents of *Withania somnifera* (L.)

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Withania preparation</th>
<th>Experimental Type</th>
<th>Disease</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>WS root extract</td>
<td>In-vivo</td>
<td>Neurodegenerative Skin carcinoma</td>
<td>↑GSH, GST, GPx, SOD Catalase and ↓ LDH in Brain, Liver and Integument tissues</td>
<td>Davis L, Kuttan G. J Ethnopharmacol 2001;75:165-168.</td>
</tr>
<tr>
<td>Regulation of Cell cycle proliferation</td>
<td>WS root extract</td>
<td>In-vivo</td>
<td>Skin carcinoma</td>
<td>↓p53, p34, cdc2</td>
<td>Singh DD, Dey CS, Bhutani KK. Phytomedicine 2001;8:492-494</td>
</tr>
</tbody>
</table>
4.9.6 Pharmacological and therapeutic uses of *Curcuma longa* (L.) and its phytoconstituents

*Curcuma longa* (CL) is a member of zingiberaceae family. Its vernacular names are haldi/haridra/manjjal (Hindi/Sanskrit/Tamil) and turmeric as a trade name. It is thought to be indigenous to the Indian subcontinent and India is the largest producer of turmeric in the world (Joe et al. 2004). Dried rhizome of CL has been widely used as a food additive (spice), preservative, colouring agent, and a traditional medicine.

![Figure 4.10: Curcuma longa (tender rhizome) Dried rhizome](image)

*Curcuma longa* preparations have been used to treat various ailments for centuries in Ayurveda (Majeed et al. 1996). Indian System of Medicine (ISM) particularly-Ayurveda, utilizes dried rhizome for the treatment of arthritis, anorexia, cough, diabetic wounds, rheumatism, sinusitis, as well as muscular, hepatic, and biliary disorders (Ammon et al. 1992). In Ayurveda, it has been described that CL preparations are taken orally to treat dyspepsia, flatulence, liver disease, urinary tract disease and as a “blood purifier.” It is also used externally for pemphigus and other skin diseases. Moreover, in traditional Chinese medicine, turmeric is used as a topical analgesic for conditions ranging from flatulence, colic, arthralgia, psychataxia, dysmenorrhea, ringworm, hepatitis and chest pain (Grant et al. 2000; Sasaki et al. 2002). In the United States, dried rhizome powder of CL is an approved food additive and is also used as an alternative treatment for disease and disease prevention (Grant et al. 2000).
Furthermore, curcumin and other phytoconstituents of CL has been reported to possess a broad spectrum of pharmacologic activities including anti-inflammatory, anti-arthritic, antioxidant, anti-mutagenic, anti-coagulant, anti-diabetic, anti-fungal, anti-viral, anti/protozoan, anti-fibrotic, anti-venom, anti-carcinogenic, anti-allergic, anti-bacterial, immunomodulating, anti-atherogenic, anti-carminative, diuretic, anti-microbial, anti-genotoxic, laxative, mosquitocidal, and anthelmintic activities (Joe et al. 2004; Chattopadhyay et al.2004; Sun et al. 2002; Araujo et al. 2001; Miquel et al. 2002; Miyakoshi et al. 2004; Roth et al. 1998; Tilak et al. 2004; Rastogi et al. 2008). Though, CL possesses numerous therapeutic applications, its anti-inflammatory and anticancer activities have recently been selected and subsequently subjected to clinical studies for evaluating the efficacy and safety of its extracts (Aggarwal et al. 2003; Chainani-Wu, 2003).

Pertaining to its broad spectrum of pharmacological activities, several clinical studies have been conducted to evaluate its anti-cancer properties for a variety of cancer (Cheng et al. 2001; Sharma et al. 2004). Sharma et al. (2004) has conducted phase-I clinical trial of rhizome extracts on colorectal cancer, they assessed glutathione S-transferase (GST) activity and the levels of an oxidative DNA adduct (M1G) and prostaglandin E2 (PGE2) in order to validate its anti-cancer properties. Moreover, they have concluded that rhizome extracts are safe for patients up to 2.2 g/day despite low oral bioavailability (Sharma et al. 2004). In-addition, another phase I study, suggested that curcumin is an effective treatment for urinary bladder cancer and is not toxic to humans when consumed not more than 8 g/day (Cheng et al. 2001). Collectively these findings suggest that curcumin appears to be safe for medicinal use; however, its efficacy for different therapeutic applications still needs to be evaluated by well-designed pre-clinical and clinical studies. Pertinent to broad spectrum of activity CL possesses; may be in-part due to the presence of major polyphenolic fraction curcuminoids, composed of curcumin, demethoxy curcumin and bis-demethoxy curcumin, which individually possess immense therapeutic potential (Majeed et al. 1996). Curcumin, the major bioactive component of curcuminoids was isolated in 1815 and the chemical structure was subsequently identified to be diferuloylmethane. In additional to
curcuminoids, other diarylheptanoids have been reported as minor constituents present in dried rhizome of CL (Park et al. 2002). Moreover, the essential oil constituents are bisabolane sesquiterpenes that include ar-turmerone, currelone, α-turmerone, β-turmerone as well as some other sesquiterpenes like zingiberene, curcumene, curcumenol, procurne, dehydrocurdione, and germacrone-13-al present in dried rhizome of CL (He et al. 1998; Hiserodt et al. 1996; Park et al. 2002; Chattopadhyay et al. 2004). Mounting evidences suggest that curcumin and other biological active constituent of CL, possess immense therapeutic potential due to its anti-oxidant, anti-inflammatory, and cholesterol-lowering properties, these three factors are the key processes involved in the pathogenesis of Alzheimer’s disease (AD) and PD (Ringman et al. 2005). Interestingly the hypothesis behind the low prevalence of AD and PD in India may be in-part due to daily consumption of CL is in wide spread, has further supported through epidemiological studies in India, suggest it has one of the lowest prevalence rates of AD and PD in the world (Chandra et al. 2001; Vas et al. 2001). Furthermore, curcumin, the major constituents of curcuminoids has been subjected to the most intensive pre-clinical and clinical research pertaining to anti-inflammatory action, cancer prevention and treatment, as well as the treatment of human immunodeficiency virus (HIV) infection (Aggarwal et al. 2003; Chainani-Wu et al. 2003; Cheng et al. 2001; Egan et al. 2004; Gescher et al. 2001; Jordan et al. 1996; Kelloff et al. 1996; Yang et al. 2005). Additionally, sesquiterpenes such as ar-turmerone in turmeric have also been reported to have hepatoprotective, mosquitocidal, and apoptosis inducing properties (Miyakoshi et al. 2004; Roth et al. 1998; Ji et al. 2004). Cumulative research evidences suggested that the antioxidant activity of *Curcuma longa* extract, curcumin and curcuminoids could be mediated through antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Sharma 2009; Ruby et al. 1995; Rastogi et al. 2008). Curcumin has been reported to act as a Michael acceptor, which can associate with glutathione and thioredoxin (Adams et al. 2005). Interestingly, reports are available stating curcumin has been found to be at least ten times more active as an antioxidant than vitamin E (Khopde et al. 1999). The reason behind its
antioxidant activity was suggested that due to the presence of phenolic and the methoxy group on the phenyl ring and the 1,3-diketone system seem to be important structural features that can contribute these effects. Moreover, relevant literature reports suggest that the antioxidant activity increases when the phenolic group with a methoxy group is bind at the ortho position (Motterlini et al. 2000). Moreover to support the various pharmacological activities exerted by curcumin Reddy and Lokesh (1994) have demonstrated that curcumin possess both phenolic and β-diketone functional groups, would be expected to have remarkable anti-oxidant and free radical scavenging activities may be in part through enhancing the activities of endogenous anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Reddy and Lokesh 1994). Consistent to it Zhang et al (1999) reported that curcumin is a potent inhibitor of oxygen radical-generating enzymes such as cyclooxygenase-2.

![Chemical structure of three components of curcuminoids](image)

Figure 4.11: Chemical structure of three components of curcuminoids

### 4.9.7 Anti-inflammatory properties of *Curcuma longa* and its phytoconstituents

Based on available reports concerning the traditional uses, clinical studies, and anti-inflammatory mechanism studies of CL and curcumin, it is evident that curcumin has great potential as a potent anti-inflammatory agent in the near future. Regarding the development of curcumin as a drug, safety appears to not be a problem due to its high dose tolerance in humans and long use as spices in daily food. However, to find out the exact mode of action, additional pre-clinical studies should be performed to extrapolate the pre-clinical findings to corroborate
with human studies. Pertaining to it various studies have been conducted to elucidate the anti-inflammatory mechanisms of curcumin and findings suggest that curcumin exerts its anti-inflammatory effect may be in-part through interactions with cytokines, lipid mediators, and eicosanoids (Joe et al. 2004). In addition mounting reports are suggesting that, variety of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) and interleukin-1β (IL-1β) can be affected by curcumin and curcuminoids and thus, downstream events involving these pro-inflammatory cytokines are attenuated (Chan et al. 1995; Ojha et al. 2012). Moreover, it is evident that many genes involved in inflammatory response initiation are regulated at the transcriptional level by the activated form of NF-κB. Expression of these inflammatory response initiation genes can therefore be inhibited by affecting the activation of NF-κB. Curcumin suppresses the activation of NF-κB by inhibiting the activity of IκB-kinases (IKKs) thereby interfering with both IKK’s activation, and IκB degradation (Brennan et al. 1998; Jobin et al. 1999; Plummer et al.1999). Reports are available that curcumin can inhibit the formation and utilization of cellular arachidonic acid, which is crucial for the generation and release of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes (Joe et al. 1997). In-addition, curcumin also serves as an inhibitor of cyclooxygenases and lipoxygenases, thus inhibiting the production of the prostaglandin E2 and the leukotrienes-B4 and C4 (Huang et al.1997; Rao et al.1995). Taken together, it is evident that active phytoconstituents of CL possess immense potential as a putative drug candidate warrants further research to validate curcumin and other constituents as a promising neuroprotective agent. Furthermore as discussed in PD pathogenesis section of the present chapter, it is evident that, though extensive research work on PD is carried out all over the world, but still no promising therapeutic intervention has developed may be due to idiopathic nature of the disease. Henceforth, the present work is an attempt to provide a new insight and therapeutic interventions of withanolides and curcuminoids over the various etiological factors brought about by oxidative injury, inflammation and mitochondrial impairment in the MPTP-induced mice model of PD.
Table 4.4: Summary of pharmacological activities of *Curcuma longa* and curcumin on the various molecular targets (*Rastogi et al. 2008; Epstein et al. 2010; Ojha et al. 2012*)

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurodegenerative disorders</strong></td>
<td>Free radical scavenger, ↓ Oxidative markers, ↓β-amyloid deposits, ↓ Inflammatory markers, ↑ Tyrosine hydroxylase</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>Induces apoptosis, Inhibits metastasis</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>↓ glucose, haemoglobin and glycated haemoglobin, ↑ antioxidant protection, ↓ Oxidative stress markers, ↑ Mitochondrial complexes</td>
</tr>
<tr>
<td><strong>Gastric diseases</strong></td>
<td>↓ growth of some Helicobacter strains, ↓ NF-κB and mitogenic response, Antifungic properties</td>
</tr>
<tr>
<td><strong>Hepatic diseases</strong></td>
<td>↓ lipid accumulation, ↓ hepatic risk biomarkers, ↓ NF-κB-dependent gene expression; ↓ inflammatory molecules expression, ↓ oxidation</td>
</tr>
<tr>
<td><strong>Ocular disease</strong></td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td><strong>Pancreatic diseases</strong></td>
<td>↓ NF-κB activation and activator protein expression, ↓ inflammatory molecules expression, ↓ caspase-3 activation, ↓ intra-pancreatic trypsin activation</td>
</tr>
<tr>
<td><strong>Atherosclerosis</strong></td>
<td>↓ LDL oxidation, Cell membrane stabilization, ↑ antioxidant plasma concentrations</td>
</tr>
<tr>
<td><strong>Respiratory disease</strong></td>
<td>↓ fibrogenesis, inflammatory markers, calcium and chloride pump alteration, Anti-asthmatic effect</td>
</tr>
<tr>
<td><strong>Intestinal diseases</strong></td>
<td>↓ lipid peroxidation, ↓ NF-κB activation, ↓ nitric oxide levels, immune function regulation, ↓ MAPK, ↓ inflammatory response</td>
</tr>
<tr>
<td><strong>Tobacco smoke-induced injury</strong></td>
<td>↓ NF-κB activation, ↓ anti-inflammatory molecules</td>
</tr>
</tbody>
</table>