1. INTRODUCTION

Parkinson’s disease (PD) or paralysis agitans is first described by James Parkinson (1817) as ‘shaking palsy’. It is a multicentric neurodegenerative disorder, characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and degeneration of non-dopaminergic systems such as noradrenergic, serotonergic and cholinergic systems (Schapira et al. 2006). The cardinal motor symptoms include bradykinesia (slowness of movement), rigidity, resting tremors (stiffness in the muscle) and dyskinesia (impaired movement), whereas non-motor symptoms such as depression, dementia, sleep deprivation and autonomic failure appear in advanced stages of PD (Meissner et al. 2011). PD is recognized as the second most common neurodegenerative disorder after Alzheimer’s disease with a prevalence of 0.1% of the global population (WHO 2004). The major concern is due to lack of early diagnosis and prognosis techniques, the cardinal clinical symptoms of PD only appears after the loss of at least 50% of dopaminergic neurons in the substantia nigra (SN) pars compacta and concomitant reduction of ≈80% dopamine in the striatum (Lang and Lozano, 1998; Deumens et al. 2002) which permits very limited treatment options against irreversible dopaminergic neuronal damage.

Furthermore, the etiology and pathogenesis of PD was suggested to be multi-factorial in nature with intricate combination of genetic and environmental components. In this context, the better understanding about the convoluted mechanisms involved in PD progression has come from 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) neurotoxin which has become the gold standard model in PD research (Dauer and Przedborski, 2003). Extensive pre-clinical and clinical research had revealed that there is intriguing association between neurotransmitters dysfunction, inflammation, glutamatergic toxicity, mitochondrial impairment, elevation of iron as well as nitric oxide levels and imbalance in antioxidants/ pro-oxidants status in the disease.
progression (Hald and Lotharius, 2005; McGeer and McGeer, 2008; Miller et al. 2009). Among the various proposed hypotheses for the etiology of PD, oxidative stress remains the strongest leading theory for the PD progression (Miller et al. 2008) and several pre-clinical and clinical studies have demonstrated the direct impact of oxidative stress on PD pathogenesis (Zhou et al. 2008; Nikam 2009). Dopaminergic neuronal cells of SNpc are particularly more vulnerable for oxidative damage due to the presence of high concentrations of iron and low concentration of endogenous antioxidants relative to the other brain regions (Sofic et al. 1991; Kedar et al. 1999).

Moreover, dual production of reactive oxygen species (ROS) derived from impaired mitochondria and dopamine (DA) metabolism, further exacerbates the oxidative stress mediated cellular damage and thus DA degeneration (Chinta and Andersen, 2008). Furthermore, ROS generation through dopamine metabolism leads to the auto-oxidation of DA with subsequent production of di-hydroxy phenyl acetic acid, DA quinone and H$_2$O$_2$ (Gotz et al. 1994). These metabolites further disrupt redox homeostasis through glutathione depletion and highly destructive hydroxyl radicals which is produced from excessive hydrogen peroxide (H$_2$O$_2$) via Fenton reaction (Graham et al. 1978; Maker et al. 1981) resulting into oxidative modifications of almost all cellular macromolecules and thus leading to dopaminergic cell death (Tse et al. 1976; Graham et al. 1978; Stokes et al. 1999; Halliwell, 2006). Meanwhile, it is evident that DA metabolism is governed by monoamine oxidase B (MAO-B) enzyme, which catalyses the oxidative deamination of DA in the substantia nigra and striatum and forms H$_2$O$_2$ as the major by-product. In addition, MAO-B is also involved in the conversion of MTPP to MPP$^+$ (1-methyl-4-phenylpyridinium) neurotoxic metabolite and it is quite evident that H$_2$O$_2$ and MPP$^+$ exert cytotoxicity over dopaminergic neurons. Henceforth, disparity in MAO-B activity also plays significant role in neurochemical alteration in the PD brain (Girgin Sagin et al. 2004; Nagatsu 2004).

Concomitantly, mounting evidences suggest that PD is a brainwide mitochondrial disease, in which reduced activity of mitochondrial complex I in substantia nigra (Schapira, 2008)
and in frontal cortex (Parker et al. 2008; Navarro and Boveris, 2009) were reported in PD patients. Moreover, analysis in peripheral tissue and skeletal tissues suggest a global reduction in mitochondrial complex I activity in PD cases (Haas et al. 1997). Furthermore, it is reported that MPP⁺ binds with mitochondrial complex-I which in-turn promotes uncoupling of oxidative phosphorylation and triggers excessive ROS generation (Fonck and Baudry 2001; Shimoke et al. 2003). Excessive ROS subsequently generates highly reactive hydroxyl radicals and peroxynitrite (Pitkanen and Robinson 1996; Raha, 2000); which collectively exacerbate DA degeneration, either through direct production of superoxide anions from increased level of free electrons and/or indirectly through dysregulation of iron (Youdim 2003; Lin and Beal, 2006).

Concurrently, impaired complex I activity leads to disruption of electron transport chain (ETC) which further exacerbate mitochondrial dysfunction and ATP depletion, which may in part causes dopaminergic cell death in the SNpc and striatum (Fleury et al, 2002; Jenner, 2003). Moreover, the dysfunction of the remaining complexes appears to be the secondary consequence of ETC disruption through complex I mediated excessive ROS generation (Lane et al. 2008). It is evident that complex I and III inhibition causes an increase in electron release from the ETC into the mitochondrial matrix, which subsequently reacts with oxygen to form deleterious free radicals such as superoxide (O₂⁻), hydroxyl ions (OH⁻) and peroxynitrite (ONOO⁻). Oxidative and nitrosative stress along with mitochondrial impairment leading to dopaminergic degeneration of the substantia nigra is believe to be the central cascade of PD progression. The elevated ONOO⁻ level acts as a powerful oxidant, which further damages mitochondrial complexes. Selective damage to complex I activity by ONOO⁻ appears to be the result of protein modifications in the form of S-nitrosation (Zhang and Dryhurst 1994; Spencer et al. 1998), whereas inhibition of Complex IV activity may be due to irreversible binding of ONOO⁻ in competition with molecular oxygen (Brown, 2001). Subsequently, excessive ONOO⁻ reacts predominately with tyrosine and cysteine residues of proteins, which are the major component of other associated mitochondrial
complexes leading to mitochondrial dysfunction through oxidative phosphorylation and further inhibits mitochondrial complex activity (Radi et al. 2002).

Along with oxidative stress and mitochondrial impairment, exacerbated inflammatory response is also reported as primary intrinsic factor in PD progression. Inflammatory responses mainly involve microglial activation, over production of cytokines and other pro-inflammatory mediators as well as the release of ROS and RNS in the PD brain (Mogi et al. 1994; Blum-Degen et al. 1995; Muller et al.1998). Moreover, up-regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were also observed in the SN of PD patients (Knott et al. 2000). These inflammatory responses together with other factors released from the dying dopaminergic neurons seem to amplify the impairment in immune responses resulting in irreversible destruction of dopaminergic neurons (Orr et al. 2002).

Taken together it is evident that multiple factors are associated with PD pathogenesis; henceforth it is apparent that the treatment strategy requires therapeutic candidates with multi-targeted pharmacological action. In this context, despite major therapeutic advancements, existing conventional drugs only provide symptomatic relief with severe side effect in long term use. Current pharmacotherapy for PD includes L-DOPA, dopamine agonists, monoamine oxidase (MAO) inhibitors, anti-cholinergic and catechol-O-methyltransferase (COMT) inhibitors (Olanow and Koller, 1998). Considering the fact that no adequate treatment interventions exist for PD cure, neuroprotective strategies which could prevent or delay the disease progression may provide promising therapeutic modalities for the prevention and treatment of PD. Pertaining to it, medicinal plants have immense potential as therapeutic candidates, since they possess potent pharmacological activities, low toxicity and economic viability. A number of Indian medicinal plants have been used for thousands of years in the Indian System of Medicine (Ayurveda) for the management of neurodegenerative diseases including Parkinson’s, Alzheimer’s, loss of memory, degeneration of nerves and other neuronal disorders. In Ayurveda, PD is defined as
“Kampavata” (Manyam, 1990) and several plant based formulations have been mentioned in Ayurvedic literature and recommended for the treatment and management of PD from time immemorial. Among several formulations, *Mucuna pruriens* seed preparations (rich source of L-dopa) are in contemporary use for the treatment of PD in India as well as other countries (Manyam and Sanchez-Ramos, 1999). Various preclinical and clinical reports suggest that *Mucuna pruriens* seeds contain L-dopa and provided long-term amelioration of Parkinson’s symptoms with reduced risk for dyskinesia in 6-OHDA rat model of PD (Lieu et al. 2010) and possessed higher anti-Parkinsonian activity when compared with synthetic levodopa (Manyam et al. 2004; Katzenschlager et al. 2004). However, current pre-clinical and clinical evidences are insufficient to evaluate the efficacy and safety of various herbal drugs used in PD management. Therefore, the scientific validation of ancient wisdom of science warrants well-designed pre-clinical studies to validate their efficacy and mode of action to justify the clinical observations.

With this pre-context, the present research work has been designed to investigate the neuroprotective effect of bioactive fractions of two revered medicinal plants of Indian System of Medicine (ISM) viz. *Withania somnifera* (L.) and *Curcuma longa* (L.) in the MPTP model.

*Withania somnifera* (WS), (Solanaceae) commonly known as Ashwagandha, has been extensively used in Ayurveda for the treatment and management of various chronic disorders including neurodegenerative diseases. The root extract of WS has shown immense medicinal properties like anti-stress, adaptogenic, hypotensive activities and also reported to be useful in the regulation of neurotransmitters. The pharmacological actions of WS root extract has been attributed to the presence of various bioactive compounds i.e. withanolides, somniferine, withanine and somniferinine. Several in-vitro and in-vivo studies demonstrated the neuro-regenerative properties of withnalide-A; protection against neuritic damage and improved synaptic plasticity in the brain (Kuboyama et al. 2002; Tohda et al. 2005). Cumulative research findings have also demonstrated the significant attenuation of oxidative stress and anti-
inflammatory responses by withanolides, somniferine and withanine treatment, resulting in neuritic regeneration, synaptic re-construction and prevention of neurochemical and neuro-psychophysiological deterioration. In addition, it was noticed that the root extract of WS is effective on the recovery of locomotive disorder along with improvement in mental health and brain aging (Singh et al. 2008). Furthermore, research findings have suggested that root extract of WS possesses antiparkinsonian effect due to its potent antioxidant, anti-peroxidative, free radical quenching and anti-inflammatory properties. (Ahmad et al. 2003). In a recent study, root extract of WS significantly inhibited haloperidol or reserpine-induced catalepsy (Kumar et al. 2006). Several recent studies suggested that WS significantly reversed the catalepsy, tardive dyskinesia and 6-hydroxydopamine elicited toxic manifestations and may offer a new therapeutic approach for the prevention and treatment of PD.

Congruently, Curcuma longa (CL) rhizome has been extensively used in India as a food ingredient, cosmetic and medicine since time immemorial. It is expected that the daily use of CL rhizome may be one of the possible factor for the low prevalence of PD in Indian population (Ojha et al. 2009/media material/7201/SfN-Chicago, IL). Curcumin, the active principle constituent of CL rhizome has multiple desirable characteristics as a neuroprotective drug candidate including, anti-inflammatory, antioxidant and anti-protein-aggregate activities. Mounting research evidences are available which suggest neuroprotective effects of curcumin are likely to be associated with its antioxidant and anti-inflammatory properties (Zbarsky et al. 2005; Chen and Le 2006; Jagatha et al. 2008). Moreover, due to its pluripotency, oral safety, long history of use, and inexpensive cost, curcumin holds great potential as a novel drug candidate for the prevention and treatment of PD.

Considering the broad spectrum of use of these two potent therapeutic candidates, the present research work has been planned to investigate the neuroprotective activity of withanolides isolated from Withania somnifera and curcuminoids derived from dried rhizome of Curcuma
*longa* in the MPTP-induced mouse model of PD. In order to explore the neuroprotective activity of the putative drug candidates, the present study has comprehensively investigated the effect of withanolides and curcuminoids over neurochemical and neurobehavioral alterations, biomarkers of PD, oxidative stress markers, mitochondrial impairment and inflammatory responses in the MPTP model. The marked effects of both the therapeutic candidates have been compared with the MAO-B inhibitor and anti-parkinsonian drug deprenyl which was used as reference standard.