Introduction
Parkinson’s Disease (PD), the second most common neurodegenerative disease, characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) region of midbrain, which results in depletion of the neurotransmitter dopamine in the striatum. The main cardinal features of PD are rigidity, resting tremor, bradykinesia and postural instability (Yadav et al., 2012). The formation of intracytoplasmic inclusions commonly called as Lewy bodies, is the main pathological symptom of this deadly disease (Gibbs and Lees, 1988). Aging, genetic and environmental factors have been implicated as the major causative factors in PD pathogenesis and progression. PD is mainly known as an aging related disease, since aging is one of the main culprits (Yadav et al., 2012). The prevalence of PD is found to be higher in the individuals of above 60 years. The genetic polymorphisms in several genes have been experimentally studied and 16 PARK loci (PARK 1 to PARK 16) have been identified to be associated with PD till date (Lesage and Brice, 2009). Mutations in these genes have been found to cause Parkinsonian syndromes, which resemble sporadic or idiopathic PD (Fahn and Sulzer, 2004).

Owing to the role of environmental toxins in the development of PD like features in humans and experimental animals, several toxins-induced PD models have been developed to investigate the molecular mechanisms of PD pathogenesis and treatment outcomes. 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), rotenone, 1,1’-dimethyl-4,4’-bipyridinium (paraquat; PQ) and manganese ethylene-bis-dithiocarbamate (maneb; MB) are the various chemicals used to recapitulate PD phenotype in the experimental rodents (Thomas and Beal, 2007; Srivastava et al., 2010). These pesticides attack on the complex I and III of electron transport chain and induce the formation of reactive oxygen species (ROS).
Epidemiological studies have revealed the higher prevalence of PD where MB and PQ in combination are immensely used as pesticides for agricultural purposes. MB and PQ act on the complexes III and I of the mitochondrial electron transport system, respectively and produce ROS, which leads to degeneration of dopaminergic neurons. Combined MB and PQ model is one of the best models of PD, owing to slow and progressive degeneration of dopaminergic neurons and induction of oxidative stress (Thiruchelvam et al., 2000; Patel et al., 2006).

Since oxidative stress plays a major role in the onset of PD phenotype and natural antioxidants, may play critical role to reduce the PD pathogenesis. Silymarin possess flavonolignan phenolics ring structure and extracted from the fruit of *Silybum marianum* [(L.) Gaertn. (Carduus marianus L., Asteraceae)], which is commonly known as milk thistle. Silymarin and its isomeric forms silibinin and isosilibinin, offer protection against chemically induced inflammation, hepatotoxicity and cancer (Upadhyay et al., 2007; Upadhyay et al., 2010; Wang et al., 2008). Silymarin prevents epithelial, prostate, bladder, lung, ovarian and breast cancers (Shalan et al., 2005; Kaur and Agarwal, 2007; Gazak et al., 2007; Svobodova et al., 2007). Silymarin easily crosses the blood brain barrier (BBB) and enters the central nervous system (Nencini et al., 2007). Silymarin is reported to protect against mitochondrial dysfunction by the prevention of mitochondrial proton leakage (Serviddio et al., 2010). Silymarin also modulates the activity of detoxification enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidise (GPx). Silymarin reduces the expression of pro-inflammatory cytokines and pro-apoptotic proteins (Wang et al., 2002; Kim et al., 2009). It did not show toxicity even at a very high concentration. Silymarin has been used as neuroprotective agents in manganese (Mn), lipopolysaccharide (LPS), 6-OHDA-, methamphetamine- and MPTP-induced neurotoxicity *in vivo* or *in vitro* system but very few reports are available.
(Chtourou et al., 2010; Wang et al., 2002; Baluchnejadmojarad et al., 2010; Lu et al., 2010). Its efficacy against MB + PQ-induced toxicity did not deciphered till now.

On the other hand, melatonin and its metabolites offer anti-inflammatory, anti-apoptotic, anti-oxidative and free-radical scavenging properties and protect against mitochondrial dysfunction (Esposito and Cuzzocrea, 2010; Galano et al., 2011; Leon et al., 2004; Mayo et al., 2005), thereby regulating multiple biological processes in the body of an organism. Melatonin reduces the expression of adhesion molecules and pro-inflammatory cytokines and regulates the expression of xenobiotic metabolizing enzymes and serum inflammatory indices (Esposito and Cuzzocrea, 2010). It is known to control the transcription, translation and catalytic activities of the preventive antioxidants, including GPx, SOD and catalase under physiological and stress conditions (Rodriguez et al., 2004). Furthermore, melatonin increases the activity of the mitochondrial complex I and complex IV, preserves homeostasis, improves respiration, enhances glutathione level, increases adenosine triphosphate (ATP) synthesis and decreases the harmful reduction in the mitochondrial membrane potential, which triggers mitochondrial transition pore opening and the apoptotic cascade (Leon et al., 2005; Srinivasan et al., 2005). Melatonin is an ideal neuroprotective agent as it can easily cross the BBB and enter the subcellular compartments, lacks toxicity as compared with many other neuroprotective agents and possesses effective combating efficacy against free radical-induced neuronal injury (Gupta et al., 2003). Although the effect of melatonin on biochemical, immunohistochemical and molecular indexes of the nigrostriatal dopaminergic neuroprotection have been reported against a few toxins-induced rodent models of PD (Acuna-Castroviejo et al., 1997; Borah and Mohanakumar, 2009; Chetsawang et al., 2009), its efficacy against MB + PQ-induced PD phenotype has not yet been deciphered.
Ability to cross the BBB, antioxidant, anti-inflammatory and anti-apoptotic nature of silymarin and melatonin prompted to investigate the effects of antioxidants against biochemical and molecular indices of MB + PQ-induced PD phenotype in mice (Nencini et al., 2007; Reiter et al., 2007). Therefore, the aim of the present study was to assess the effect of antioxidants (silymarin and melatonin) on neurodegeneration in MB + PQ-induced PD phenotype in mouse and also to assess the involvement of oxidative stress, toxicant responsive, apoptotic pathways in PD pathogenesis.

**OBJECTIVES**

The major objectives of this study are:

1. To investigate the effect of antioxidants (silymarin/melatonin) on motor functions/neurobehavioral parameters in animals and tyrosine hydroxylase immunoreactivity and dopamine level in nigrostriatal tissue of control and MB + PQ-induced PD phenotype in mouse

2. To investigate the effect of antioxidants (silymarin/melatonin) on the indices of pro-oxidants and antioxidants in nigrostriatal tissue of control and MB + PQ-induced PD phenotype in mouse

3. To investigate the effect of antioxidants (silymarin/melatonin) on global gene expression profile including the genes related to oxidative stress, xenobiotics metabolism and apoptosis in nigrostriatal tissues of control and MB + PQ-induced PD phenotype in mouse