EFFECT OF IBUPROFEN ON CYPERMETHRIN-INDUCED ALTERATIONS IN THE MITOCHONDRIAL FUNCTION, DENDRITIC ARBORIZATION AND SPINE DENSITY ASSOCIATED WITH PARKINSONISM

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Babu Banarasi Das University
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by

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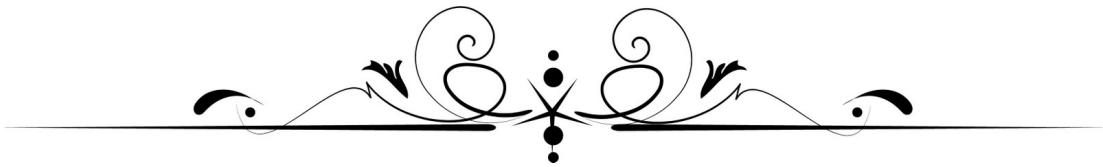
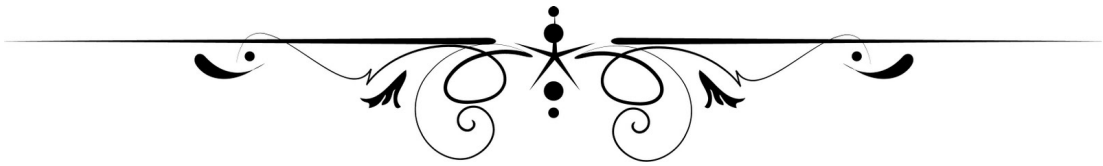


Dedicated to
My Beloved Parents



CERTIFICATE OF THE SUPERVISOR/CO-SUPERVISOR

This is to certify that the thesis, entitled **"Effect of ibuprofen on cypermethrin-induced alterations in the mitochondrial function, dendritic arborization and spine density associated with Parkinsonism"** submitted by Ms. Pratibha Tripathi for the award of Degree of Doctor of Philosophy by Babu Banarasi Das University, Lucknow is a record of authentic work carried out by her under the supervision of Prof. (Dr.) Lakshmi Bala and Co-supervision of Dr. M. P. Singh. To the best of our knowledge, the matter embodied in this thesis is the original work of candidate and has not submitted elsewhere for the award of any other degree or diploma.

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DECLARATION BY THE CANDIDATE

I, hereby, declare that the work presented in this thesis, entitled **"Effect of ibuprofen on cypermethrin-induced alterations in the mitochondrial function, dendritic arborization and spine density associated with Parkinsonism",** in fulfilment of the requirements for the award of Degree of Doctor of Philosophy of Babu Banarasi Das University, Lucknow in an authentic record of my own research work carried out under the supervision of Prof. (Dr.) Lakshmi Bala and Co-supervision of Dr. M. P. Singh. I also declare that the work embodied in the present thesis is my original work and has not been submitted by me for any other Degree or Diploma of any university or institution.

Date: **(Pratibha Tripathi)**

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(Pratibha Tripathi)

PREFACE

Parkinson's disease (PD) is a slow and progressive neurological disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra and subsequent reduction in the dopamine level and motor activities. Aging, genetic factor and environmental exposure to pesticides and metals are the major contributors of PD. Cypermethrin, a pyrethroid pesticide induces PD like symptoms in experimental animals. However, the mechanism of cypermethrin induced PD is yet not fully known. The postnatal animals are more susceptible for pyrethroid induced toxicity as compared with the adults because pesticides hamper the brain development leading to some subtle alterations that could enhance the susceptibility upon re-exposure. While, cypermethrin induces oxidative stress, mitochondrial dysfunction, and dopaminergic neuronal cell death, cypermethrin mediated neuroinflammation and terminal loss of dopaminergic neurons are not investigated.

Present study entitled "Effect of ibuprofen on cypermethrin-induced alterations in the mitochondrial function, dendritic arborization and spine density associated with Parkinsonism" was investigated to assess the effect of ibuprofen, an anti-inflammatory drug against cypermethrin induced alterations on the synapses and dendrites under the influence of neuroinflammation. The effect of ibuprofen, a non-steroidal anti-inflammatory agent was monitored by performing the biochemical and histopathological parameters in the nigrostriatal tissue of the brain of cypermethrin exposed rats along with control.

Cypermethrin treatment resulted in the significant decrease in the number of neuronal nuclei-tyrosine hydroxylase (NeuN/TH) positive cells and increased the number of activated microglia in the substantia nigra. Moreover, striatal dopamine

and nigral tyrosine hydroxylase (TH-immunoreactivity) were reduced after cypermethrin exposure. Cypermethrin also degenerated dopaminergic synapse and reduced the number of striatal dendrites and dendritic spines. Furthermore, cypermethrin reduced complex I activity and expression of apoptotic, inflammatory and synaptic proteins.

On the other hand, ibuprofen rescued from the deleterious effects of cypermethrin since it was found to increase the number of NeuN/TH positive cells and decrease the number of activated microglia. Ibuprofen also increases the striatal dopamine and TH-immunoreactivity in cypermethrin exposed animals. Ibuprofen also protects from the cypermethrin induced reduction in the number of dendrites and dendritic spines of the medium spiny neurons. The altered expression of apoptotic, inflammatory and synaptic proteins was shifted towards normalcy when animals were treated with ibuprofen.

The thesis is divided into five chapters. **Chapter I** describes the review of the literature, containing the detailed information about the etiology, pathological features, dopamine metabolism, animal models, diagnosis and treatment of PD. This chapter also discusses about mitochondrial dysfunction, inflammation, oxidative stress, ion channels and microglial activation in PD. **Chapter II** deals with the aims and objectives of the study. **Chapter III** describes the effect of ibuprofen on the basic Parkinsonian features, such as NeuN/TH-immunoreactivity, dopamine level, microgliosis and mitochondrial dysfunction of the cypermethrin-induced rats. **Chapter IV** highlights alterations in the morphology and number of the striatal dendrites and dendritic spines of the cypermethrin after exposure and rescuing potential of ibuprofen. Ibuprofen mediated alleviation in the cypermethrin induced

changes in the apoptotic, inflammatory and synaptic proteins are described in **chapter V**. Moreover, it also deals with the protective efficacy of ibuprofen as those variables in cypermethrin treated rats. Overall the results of the study are summarized in a separate section.

Ibuprofen mediated protection against cypermethrin induced neurotoxicity is described in **Chapter III** to **Chapter V** and published.

1. Ibuprofen Protects from Cypermethrin-Induced Changes in the Striatal Dendritic Length and Spine Density. **Pratibha Tripathi**, Ashish Singh, Lakshmi Bala, DK Patel, MP Singh (2018). Mol Neurobiol. 55(3):2333-2339.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Bax	Bcl-2–associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra large
BSA	Bovine serum albumin
COX-2	Cyclooxygenase-2
DA	Dopamine
DAT	Dopamine transporter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribo nucleic acid
DPX	Dibutyl phthalate xylene
EDTA	Ethylene diamine tetra acetic acid
GABA	Gamma-aminobutyric acid
GST	Glutathione-S-transferase
IFN-γ	Interferon- γ
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
LRRK2	Leucine-rich repeat kinase 2
MAPK	Mitogen activated protein kinase
MB	Maneb
MSNs	Medium spiny neurons
min	Minute
MMP-3	Matrix metalloproteinase-3
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAD	Nicotinamide adenine dinucleotide
NeuN	Neuronal nuclei
NSAIDs	Non-steroidal anti-inflammatory drugs
3-NT	3-Nitrotyrosine

6-OHDA	6-Hydroxydopamine
PARK	Parkinson disease associated gene
PBS	Phosphate buffer saline
PD	Parkinson's disease
PINK1	PTEN induced putative kinase 1
PQ	Paraquat
PSD-95	Postsynaptic density protein-95
PTEN	Phosphatase and tensin homolog
RES	Reserpine
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulphate
SNpc	Substantia nigra pars compacta
SYN	Synaptophysin
TH	Tyrosine hydroxylase
VGCC	Voltage gated calcium channels
VMAT2	Vesicular monoamine transporter2

CHAPTER 1

REVIEW OF LITERATURE

CHAPTER 1

Review of Literature

1.1. Parkinson's disease (PD)

PD is a slow progressive neurological disorder of unclear pathogenesis affecting mainly the elderly. The motor symptoms of PD results from the selective loss of dopaminergic neurons in the substantia nigra and loss of their axon terminals projected in the striatum **(Abeliovich and Gitler, 2016)**. The complex etiology of the disease is mainly caused due to the genetic factors, environmental exposure or both in combinational form **(Terzioglu *et al*., 2008)**. The key symptoms of the PD are the resting tremor, bradykinesia, postural instability and rigidity. PD was first described by a British physician, James Parkinson, in his article "An Essay on the Shaking Palsy" as "paralysis agitans" in 1817 **(Parkinson, 2002)**. French neurologist Jean-Martin Charcot coined the name "Parkinson's disease" in 1867 **(Goetz, 1986; Lees, 2007)**. Many other non-motor symptoms have been recognized like sleep disturbance, cognitive impairment, depression and olfactory dysfunctions are also common disabling manifestations of the disease. More than 1% population affected due to the PD over the age of 60 years. In children and adults, the genetic form of PD is reported. The clinical symptoms appear upon 60-70% degeneration of dopaminergic neurons that results in the dopamine depletion in the nigrostriatal system **(Lang and Lozano *et al*., 1998)**.

1.2. Symptomatic features

Among two main features of PD, clinical symptoms are categorized into the motor and non-motor symptom. Clinical symptoms are further divided into the cardinal and secondary features depending upon their time of appearance. Resting tremor, bradykinesia, muscular rigidity and postural instability are the cardinal features **(Jankovic, 2008; Ur Rasheed *et al*., 2015)**. Dysarthria, micrographia, dysphagia, hypomimia, dystonia, shuffling gait, sialorrhoea, festination, sleep disorders, glabellar and cognitive neurobehavioral/sensory abnormalities are the secondary features of PD patients **(Jankovic, 2008)**. Another one is the anatomical and pathological symptom in which the damaged neurons show biochemical and structural alteration. In the surviving dopaminergic neurons accumulation of cytoplasmic inclusion occurs known as Lewy bodies and these are the cytological characterization of PD **(Forno, 1996)**. Lewy bodies are made up of insoluble fibrous proteins such as α-synuclein and ubiquitin **(Olanow and Tatton, 1999; Fahn and Sulzer, 2004; Yadav *et al.*, 2012a)**.

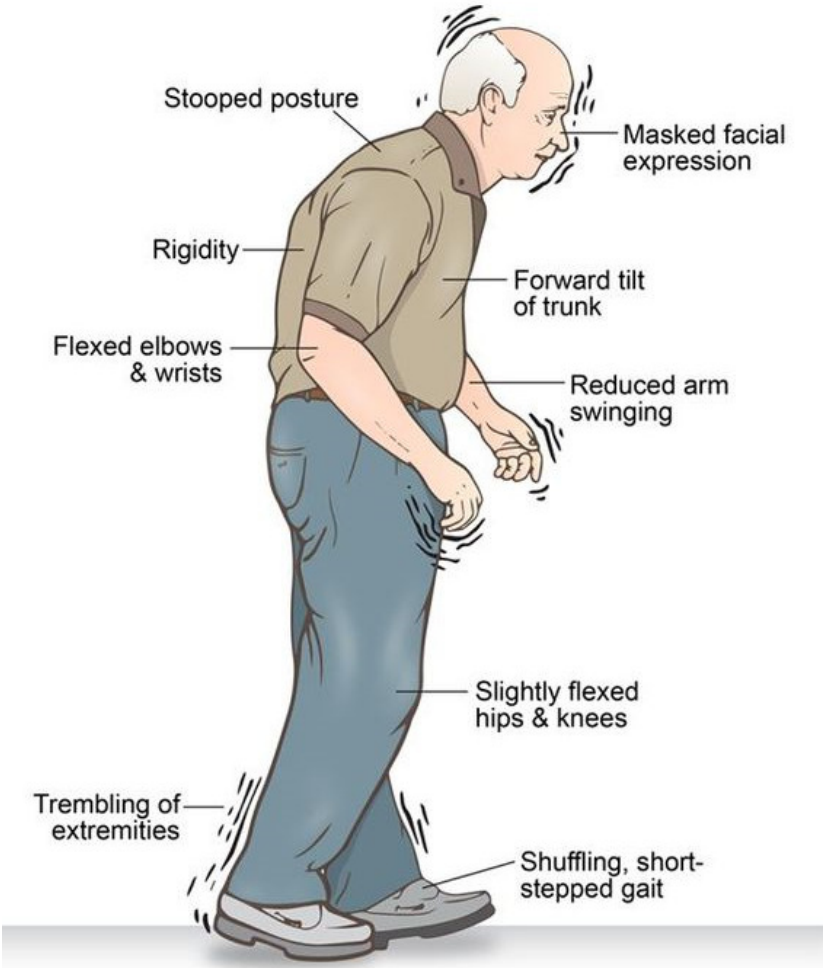


Figure 1.1: Typical appearance of a PD patient (Source: https://twitchywoman.com/2016/10/24/breast-cancer-vs-parkinsons)

1.3. PD incidences

PD is an age related disease and occurs after the age of 60 years. However, early onset of disease has also been reported and occurs at the age of 40 years **(Van Den Eeden *et al.*, 2003)**. Males are more susceptible to PD as compared to females. The major reason for such inconsistency may be due to the hormonal inequality or low level of estrogen in males **(Van Den Eeden *et al.*, 2003)**. Another reason for the prevalence of disease in males may be the presence of PD susceptibility genes that are located on the X chromosome. However further study is still needed to prove the hypothesis **(Wooten *et al.*, 2004)**.

1.4. Pathophysiology of PD

The movement and body posture are mainly regulated by the basal ganglia and the mid brain region of the brain **(Takakusaki and Okumura, 2008; Fitzgerald, 1996)**. The major parts of the basal ganglia circuit that maintain the motor coordination is the caudate and putamen, external and internal segment of the globus pallidus, subthalamic nucleus and substantia nigra **(Lang and Lozano, 1998)**. The direct and indirect pathways are regulated by the dopamine for the motor function **(Nishi *et al.,* 2011)**. In the normal condition, the ratio of acetylcholine and dopamine remains to be fixed however in case of PD the level of dopamine gets reduced **(Calabresi *et al.*, 2006)**. Due to the dopamine depletion, the indirect pathway gets hyper-activated while the direct pathway remains hypo-activated. Reduced motor activity, muscular rigidity, resting tremor develops due to the depletion of dopamine **(Lang and Lozano, 1998; Nicholson *et al.,* 2002)**. The characteristic feature of PD is the death of dopaminergic neurons and Lewy body formation in the adjacent neurons **(Dauer and Przedborski, 2003; Dickson, 2007)**. Excess of free radical generation and depleted cellular antioxidant defense machinery induces the oxidative stress **(Bains and Shaw, 1997)**. PD pathogenesis could also be indicated by the microglial activation **(Gerhard *et al.*, 2004; Ouchi *et al.*, 2005)**. Depleted dopamine level in the striatum and reduced TH-immunoreactivity is also responsible for the pathogenesis of PD **(Duan *et al.*, 2005)**. In experimental models, it has been reported that the reduced level of anti-apoptotic genes and increased expression of apoptosis and necrotic factors are responsible for the nigrostriatal dopaminergic neurodegeneration **(Nagatsu and Sawada, 2007)**.

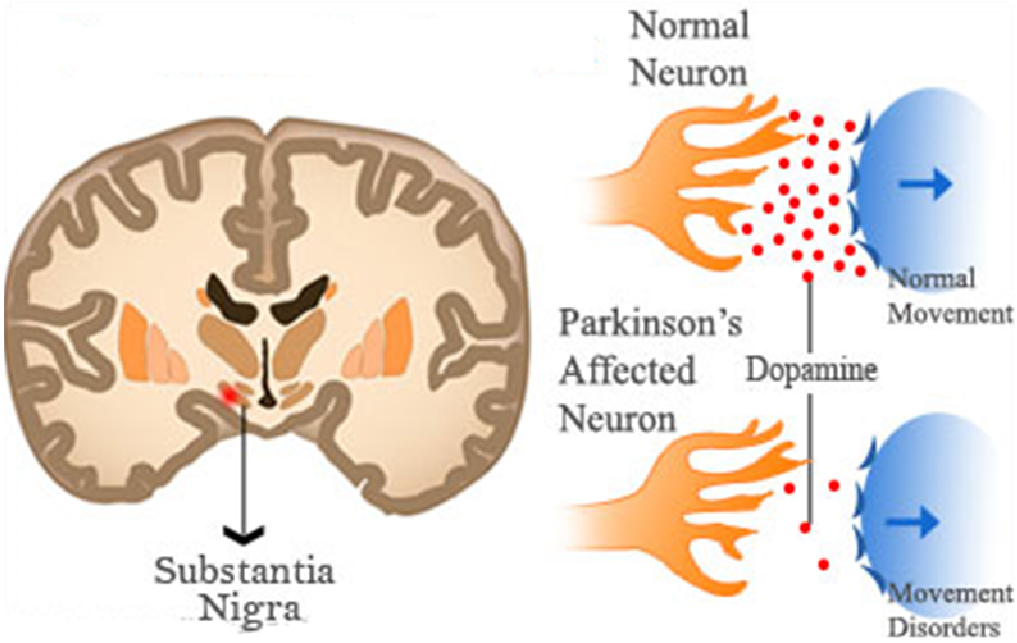
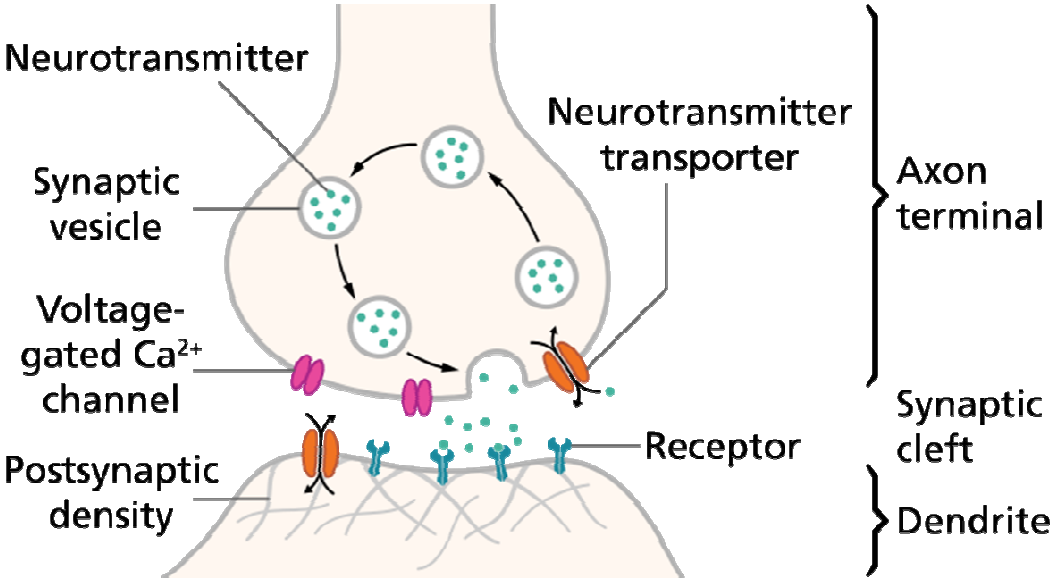


Figure 1.2: Diagrammatic representation of pathophysiological characteristics of PD (Source:http://www.medindia.net/patients/patientinfo/parkinsonsdisease.htm)

1.4.1. Loss of striatal dopaminergic terminals

It is a big unanswered question in recent years that from where PD begins and what are the preliminary events. The answer of this question is of course critical. Many years back Hornykiewicz discovered that dopamine depletion takes place in PD **(Burke *et al.*, 2013)**. He proposed the dying-back degeneration in case of PD as the striatal terminals are comparatively enriched with dopamine transporters than the nigral cell bodies **(Hornykiewicz, 1998; Burke *et al.*, 2013)**. Earlier studies have revealed that the loss of dopaminergic neurons in the substantia nigra leads to the loss of dopaminergic terminals in the striatum and gives the early signs of PD pathology **(Burke *et al.*, 2013)**. The data from earlier studies have revealed that depending upon the duration of disease onset, more pronounced deterioration of dopaminergic markers takes place in the striatum **(Burke *et al.*, 2013)**.



(Figure 1.3: Diagrammatic representation of synapse)
(Source:https://commons.wikimedia.org/wiki/File:SynapseSchematic_en.svg)

1.4.2. Why dopamine axons are so vulnerable?

There have been many studies that dopaminergic neuronal loss occurs in the substantia nigra and terminal loss occurs in the striatum during the PD progression. Dopamine is more sensitive to oxidize in the cytoplasmic environment. These processes lead to the generation of free radicals and interfere with many cellular processes such as bioenergetics, microtubule stability, trafficking of organelles and autophagy. Two independent groups have reported in their data that the mitochondria of dopaminergic neurons are only 40% in size than the mitochondria present in the non-dopaminergic neurons. In addition the mitochondria present in the dopaminergic axons are three times slower than the axons of non-dopaminergic (**Kim-Han *et al.*, 2011; Liang *et al.*, 2007**). Due to the comparatively more extensive terminal fields of dopaminergic neurons it is more difficult to getting mitochondria where they are required at the time upon getting challenged. Therefore, any of these process, microtubule disassembly, mitochondrial dysfunction, autophagy induction and

reactive oxygen species (ROS) production can cause the axonal trafficking problems, dysfunction and ultimately death of neurons **(Matsuda *et al.* 2009; Burke *et al.*, 2013)**.

1.4.3. Dopamine metabolism

Dopaminergic neurons are present in various regions of brain, like substantia nigra, ventral tegmental area, hypothalamus, infundibular nucleus and zona incerta **(Dahlström and Fuxe, 1964)**. The central nervous system of mammals is abundantly supplied with dopamine through the nigrostriatal pathway. In the nigrostriatal pathway the dopamine is synthesized by the dopaminergic neurons in the substantia nigra and supplied to striatum through their terminals **(Fahn and Sulzer, 2004)**. The nigrostriatal dopaminergic pathway gets affected during the PD. Tyrosine is a semi essential amino acid that is responsible for the synthesis of dopamine. The first step of dopamine synthesis is the conversion of L-tyrosine into the L-3, 4-dihydroxyphenylalanine (L-DOPA) in the presence of enzyme, tyrosine hydroxylase (TH). In the presence of enzyme, L-amino acid decarboxylase, dopamine synthesis takes place from L-DOPA. The synaptic vesicles sequester the cytosolic dopamine for storing and rescuing them from oxidation **(Sulzer *et al.*, 2000; Caudle *et al.*, 2007)**. The synaptic cleft contains the packed dopamine and supplied by binding with their transporters in postsynaptic terminals. The catabolic products of dopamine are inactive metabolites like, 3-methoxytyramine and homovanillic acid formed by monoamine oxidase (MAO), aldehyde dehydrogenase and catechol-o-methyl transferase (COMT). In the absence of proper metabolism, the excess dopamine gets auto-oxidized to produce free radicals, responsible for damage of neurons **(Sulzer and Zecca, 2000)**.

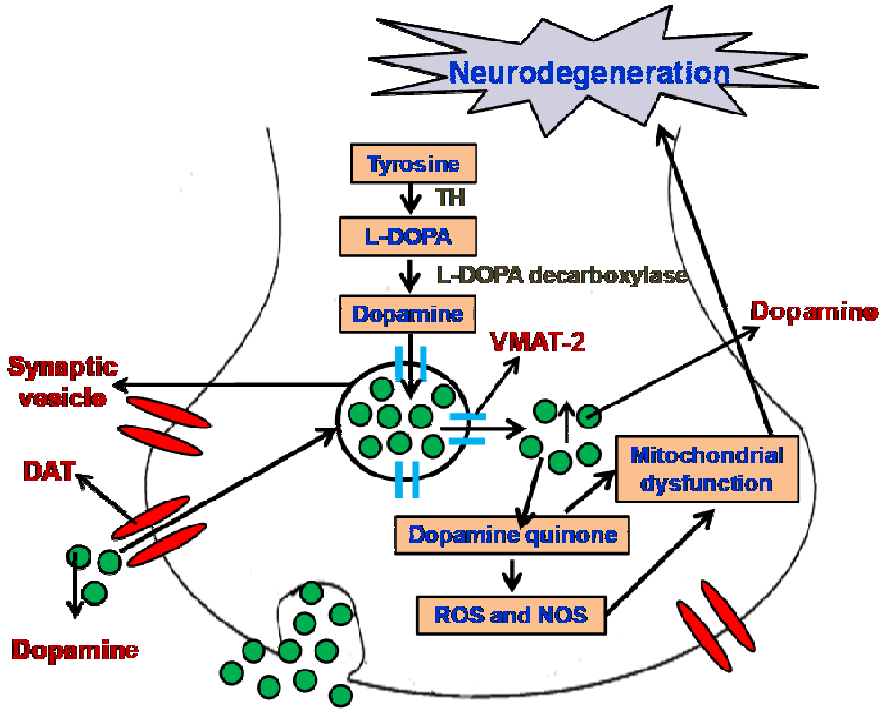


Figure 1.4: Diagrammatic representation of dopamine metabolism;VMAT2(vesicular monoamine transporter2); DAT(dopamine transporter); TH (tyrosine hydroxylase); ; (Source: Lotharius and Brundin, 2002)

1.4.4. Role of dopamine in disparity of striatal spines in PD

Dopamine is a neurotransmitter, abundantly found in the striatum and contributes in the normal function of basal ganglia. The dopaminergic and glutamatergic inputs are received by the spines present on the dendritic arbor of the medium spiny neurons in the striatum **(Villalba and Smith, 2013)**. Based on direct and indirect pathway the spiny neurons of the striatum can be divided into the dopamine D1 receptor (DA-D1R) rich neurons (direct pathway) and dopamine D2 receptor (DA-D2R) rich neurons (indirect pathway) **(Cahill *et al.*, 2014)**. The dopaminergic neuron projections present in the substantia nigra and the dorsal striatum receives dopamine from terminals **(Gerfen *et al.*, 1987; Lynd-Balta and Haber, 1994 a, b)**. Upon

dopamine depletion, the medium spiny neurons of striatum gets into the hyperexcitable state because of the dysregulated release of glutamate from corticostriatal afferents, leads to the loss of dendritic spines **(Naskar *et al*., 2013)**. In PD the loss of dendritic spines of medium spiny neurons in the striatum is reported **(Villalba and Smith, 2013)**.

1.4.5. Loss of Striatal spines in rodent models of PD

Studies have been done to found the connection between the striatal spine loss and PD. The results from the experimental studies of 6-hydroxydopamine (6-OHDA) intoxicated rats showed 20% loss in the striatal spines. In the animal models for striatal spine loss it was the first confirmation **(Ingham *et al.*, 1989)**. There is a close association between denervation pattern in the striatum and degree of the loss of striatal spines **(Villalba and Smith, 2013)**. Findings from the studies on 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) model of non-human primate also supported the fact that the loss of striatal spines corresponds to the dopaminergic denervation pattern in PD. Altogether these experimental studies and post-mortem data provide evidences that the striatal spine loss is governed under the influence of nigrostriatal dopaminergic neurodegeneration **(Villalba and Smith, 2013)**.

1.4.6. Genetic causes and terminal degeneration in relation with PD

Previous studies have experimentally proved that the presynaptic terminals of neurons are enriched with α-synuclein pathology. Retrograde progression of disease was evident from the α-synuclein pathology pattern **(Burke et al., 2013)**. Earlier experiments have demonstrated that the aggregation of α-synuclein started at presynaptic terminal and concomitantly extends towards neuron soma and also leads

to the deterioration of synaptic proteins **(Volpicelli-Daley *et al*., 2011)**. The leucine rich repeat kinase (LRRK2) gene mutations are commonly known for the genetic cause of PD **(Greggio and Cookson, 2009)**. To study the genetic form of PD, the experimental study was conducted in the mouse model and it was concluded the role of axons in disease progression. The neurite growth and integrity are regulated by LRRK2 and this possibility was supported by other evidences **(Paglini *et al*., 1998)**. Based on the site where PD pathology was found predominantly and autosomal dominant genetic forms of PD support the fact that the dysregulation of axons indicates the early events of PD **(Burke *et al*., 2013)**.

1.5. Risk factors for PD

Aging is the primary cause of PD but there are several risk factors such as genetic susceptibility, environmental factors including pesticides exposure are the major factors that contribute for the PD.

1.5.1. Age

PD is an elderly neurological disorder thus in young individuals its prevalence is very low except few cases of PD in juveniles **(Aschner, 2000; Giasson and Lee, 2001; Shastry, 2001; Kanthasamy *et al.*, 2005)**. The risk of PD increases with the advancement of age in older people and genetic or environmental factors could play a pivotal role for it. Further several animal studies have supported the fact that with the increasing age the risk of PD also increases **(Irwin *et al.*, 1992)**.

1.5.2. Gender

Although it is reported that the males are at higher risk of PD occurrence still the gender biases is the topic of debate. Due to the presence of estrogen in females, they

are comparatively at lower risk than males. Estrogen could provide neuroprotection by mitogen activated protein kinase (MAPK) activation and Bcl-2 associated X protein (Bax) modulation. However further study is still required to prove the hypothesis **(Wooten *et al.*, 2004)**.

1.5.3. Environmental factors

Environmental factors include all those influences that are originated from outside the genome, substances that we breathe from the air and few metabolic changes that are induced by the activities we perform. The environmental factors such as the contaminated air, water and food are taken by organisms in their day to day life also influence the biological activity of the organism. The biochemical activities of blood brain barrier permeability, microgliosis, inflammatory molecules and cell death due to the abnormal cell signaling are directly or indirectly affected by environmental factors **(Horowitz *et al.*, 2010)**. The risk of PD may be increased due to the environmental factors like heavy metals, rural area accommodations, frequent exposure of herbicides and pesticides in the agricultural field, toxins produced by microbes and polluted drinking water **(Singh *et al.*, 2007)**.

1.5.4. Genetic factors

In case of PD, the genetic model is very important in order to understand the genetic etiology of PD and to develop new therapeutic interventions. The genetic variations among the people are very crucial in developing neurological disorders like PD. So far total eighteen Parkinson disease associated genes (PARK) loci have been characterized **(Klein and Westenberger, 2012)**. The inheritance of PARK genes occurs in the pattern either autosomal recessive or autosomal dominant and often

causes the early age onset of disease **(Bezard and Przedborski, 2011)**. The familial form of PD is related to the mutations in gene. In rodents and lower organisms by genetic manipulations, many genetic models of PD have been developed. On the basis of occurrence pattern, genetic model of PD are categorized into the autosomal dominant and autosomal recessive types **(Dawson *et al.*, 2010)**.

1.5.5. Lifestyle factor

The risk of PD is also influenced by the nutritional habit and day to day life style. Nicotine and caffeine are used by people in their daily life. There is a strong association between the uses of tobacco in a dose dependent manner and risk of PD. However, it is not yet clear whether it because of consequence of tobacco use or constituent of tobacco is responsible for that **(Ritz *et al.*, 2007)**. Nicotine provides neuroprotection by altering the molecular and cellular mediators in the dopaminergic system **(Quik *et al.*, 2008)**. Neuroprotection may also offer by a standard cup of coffee that contains 100 mg of caffeine. Experimental studies have shown that an average amount of 200-250 mg/day/person may reduce the risk of PD. Some studies have also shown an inverse association of caffeine and nicotine with the PD risk **(Ascherio *et al.*, 2001; Singh *et al.*, 2008; Yadav *et al.*, 2012b)**. Mediterranean diet includes high intake of vegetables, fruits, unsaturated fatty acids and fishes which are responsible for minimizing the PD risk. Other foods like red meat, processed food and animal fat are found to elevate the chances of PD **(Barichella *et al.*, 2009)**. Sluggish nature, sedentary lifestyle and not having physical exercise may also contribute to the risk of PD.

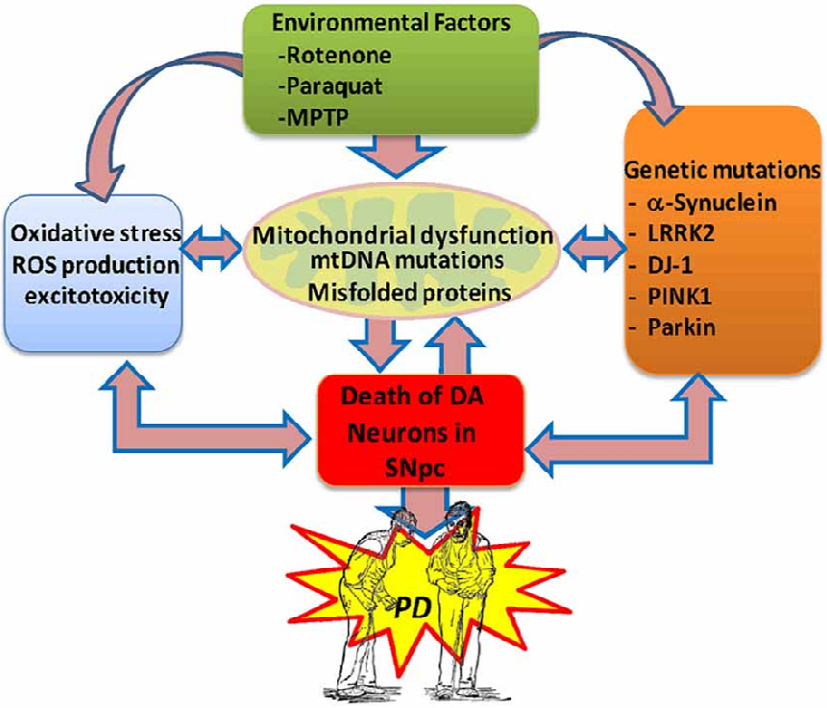


Figure 1.5: Factors responsible for PD
(Source: Barreto et al., 2015)

1.6. Rodent models

In order to understand the etiology and molecular pathogenesis of PD, extensive efforts have been made **(Dawson *et al.*, 2002)**. The exact molecular mechanism of the disease, early diagnosis and permanent treatment of the disease is still not known. To investigate the underlying mechanism of the disease some animal models have been developed so far **(Betarbet *et al.*, 2002)**. The study on animal models has been suggested that the dopamine depletion in the striatum is responsible for the motor impairment **(Singh *et al.*, 2012b)**. Studies on the animal model have suggested that the neural stem cell transplantation is also an effective therapeutic tool for the treatment of PD **(Dawson *et al.*, 2002; Steindler, 2007)**. The common cardinal symptoms of the sporadic PD such as striatal dopamine depletion, nigral degeneration of dopaminergic neurons and motor abnormalities had been found in

the 6-hydroxy dopamine model (6-OHDA) and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) model **(Porras *et al*., 2012; He *et al*., 2001)**. PD does not become visible naturally in animals, as it is a human disease. Therefore successful therapeutic strategies could not be developed on the basis of experimental animal studies **(Terzioglu and Galter, 2008; Waldmeier *et al.*, 2006; Lane and Dunnett, 2008)**.

1.6.1. 6-hydroxy dopamine (6-OHDA)

For the first time 6-OHDA model of PD was established **(Tieu *et al.*, 2011)**. 6-OHDA is reported to cause the selective loss of catecholaminergic neurons. 6-OHDA is the hydroxylated analog of the naturally present neurotransmitter dopamine **(Tranzer and Thoenen, 1973)**. 6-OHDA is naturally present in the brain of human and showing high affinity to the dopamine transporter, therefore it is extensively used for the study of the PD pathophysiology. 6-OHDA quickly oxidizes to form hydrogen peroxide, superoxide, hydroxyl radicals and much more **(Cohen *et al.*, 1974)**. In the 6-OHDA exposed animals, the quantifiable lateral motor movement occurs opposite to the lesion side and are used extensively for the study of the neuroprotective efficacy of the various pharmacological agents to rescue from PD **(Ilijic *et al.*, 2011; Tieu *et al.*, 2011)**. 6-OHDA is not considered as an appropriate model of PD because it is a nonenvironmental toxin and it cannot be administered through invasive route **(Tieu *et al*., 2011; Betarbet *et al.*, 2002)**.

1.6.2. 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)

In 1982, MPTP was accidentally discovered when a group of young drug addicts developed sub-acute onset of severe Parkinsonism in California, incident by the

contaminant present in the heroin meperidine **(Langston *et al.*, 1983)**. Later on, this contaminant was identified as MPTP. The systemic administration of the MPTP in mice and primates subsequently developed the severe features resembling the sporadic PD. MPTP exposure causes the reduction in the biosynthesis of ATP and free radical generation by inhibiting the complex-I of the electron transport chain in the nigral dopaminergic neurons **(Langston *et al.*, 1984)**. MPTP is lipophilic in nature and easily crosses the blood brain barrier. MPTP itself is not toxic but its oxidized form, 1-methyl-4-phenyl pyridinium (MPP^+) is toxic **(Sadek *et al.*, 2014)**. MPTP led to the interest in the study of the role of environmental toxins in the development of Parkinsonism and particularly to those that have a similar structure as the MPTP metabolite MPP^+ **(Thiruchelvam *et al.*, 2000)**. Since then many epidemiological and toxicological studies have been published showing the role of pesticide and its possible mechanism involved in the incidence of PD.

1.6.3. Maneb and paraquat model

Paraquat (PQ) is a bipyridyl derivative having similar structure as the MPP^+, a metabolite of MPTP **(Miller, 2007)**. Paraquat is extensively used pesticide causes oxidative stress. The toxic mechanism of the pesticide is directly or indirectly related to the reactive oxygen species **(Somayajulu-Nitu *et al.*, 2009; Patel *et al.*, 2007; Miller, 2007)**. In experimental animals, upon paraquat exposure, most of the biochemical and neuropathological features of human Parkinsonism could develop **(Patel *et al.*, 2007; Gupta *et al.*, 2010)**. In combination with other pesticides such as maneb (MB) the exposure of paraquat occurs therefore in mice dual pesticide model has been developed **(Thiruchelvam *et al.*, 2000; Cicchetti *et al.*, 2005)**. Exposure to

maneb and paraquat in combination causes the pronounced loss of motor activity in the exposed animals **(Thiruchelvam *et al.*, 2000)**.

1.6.4. Rotenone model

Rotenone is a pesticide used from past few decades for the plants **(Cabras *et al.*, 2002)**. In the similar fashion as MPTP, rotenone also inhibits the mitochondrial complex-I **(Langston *et al.*, 1983; Cabras *et al.*, 2002)**. Rotenone causes the nigral dopaminergic neurodegeneration in the substantia nigra and also results in the Lewy body formation, the pathological feature of PD **(Xiong *et al.*, 2009)**. Due to the very short half life of rotenone, there is controversy over the potential of rotenone for causing PD. However, in rats, the limited exposure of rotenone causes the progressive neurodegeneration, mimicking the changes as in human PD **(Tanner *et al.*, 2011)**.

1.6.5. Reserpine and methamphetamine model

Reserpine (RES) was used to induce PD in rabbit and it was hypothesized for the first time that in the striatum dopamine depletion occurs and it results in the motor abnormality in the PD patients and levodopa administration recovers the deficiency **(Carlsson *et al.*, 1957)**. In the similar fashion as RES, methamphetamines (METH) also cause the depletion of the dopamine in the striatum and persuade PD like symptoms without any change in the dopaminergic neurons **(Tieu *et al.*, 2011; Betarbet *et al.*, 2002)**. RES and METH model did not show all the clinical features of PD, therefore, it was only accepted as a dopamine depletion model, not as a true model of PD **(Tieu *et al.*, 2011; Betarbet *et al.*, 2002; Yadav *et al.*, 2012a)**.

1.6.6. 3-Nitrotyrosine model

Oxidative stress is responsible for the production of nitrated form of tyrosine, 3-nitrotyrosine (3-NT), in many neurological disorders **(Mohiuddin *et al.*, 2006; Pacher *et al.*, 2007; Beal, 2002)**. Oxidative stress causes the damage of deoxyribose nucleic acid (DNA) and protein in PD patients **(Burney *et al.*, 1999)**. The stereotactic injection of 3-NT in the striatum induces the symptoms like motor deficits and loss of TH-immunoreactivity **(Mihm *et al.*, 2001)**.

1.6.7. Metals

The higher or lower level of metals could be responsible for the serious neurological disorders **(Montgomery *et al.*, 1995)**. The heavy metal contaminants could be present in the food and drinking water. Thus these metals increase the risk of PD in the industrial areas. Epidemiological and experimental studies have proven the role of heavy metals in the PD pathogenesis. The neurotoxicity could be enhanced by these metals alone or in combination of other metals or chemicals **(Peng *et al.*, 2008)**. Some metals have well established role in the brain neurotoxicity and PD pathogenesis **(Dexter *et al.*, 1991)**.

1.6.8. Lipopolysaccharide model

Lipopolysaccharide (LPS) is produced by the gram negative bacteria and after their insertion they cause the dopaminergic neurodegeneration **(Stewart *et al.*, 2006; Qin *et al.*, 2007; Castano *et al.*, 1998)**. Once inside the neuron, LPS induces neuroinflammation by producing the nitric oxide and free radicals in addition to the microglial activation **(Barron, 1995; Block *et al.*, 2007; Liu, 2006; Herrera *et al.*, 2000)**. Immunohistochemical analysis in the PD patients and animal models showed

the microgliosis **(McGeer and McGeer, 2004)**. Since the LPS induces neuroinflammation thus it could not be taken as a suitable model for the study of PD pathology.

1.6.9. Dual models

In order to describe the role of environmental factors in genetically susceptible PD models the dual/combinational/fusion/two-hit models have been developed **(Yadav *et al.*, 2012a)**. In the dual model of PD several approaches have been applied to produce key symptoms of the disease **(Gao and Hong, 2011; Manning-Bog and Langston, 2007; Gao *et al.*, 2011; Yadav *et al.*, 2012a)**.

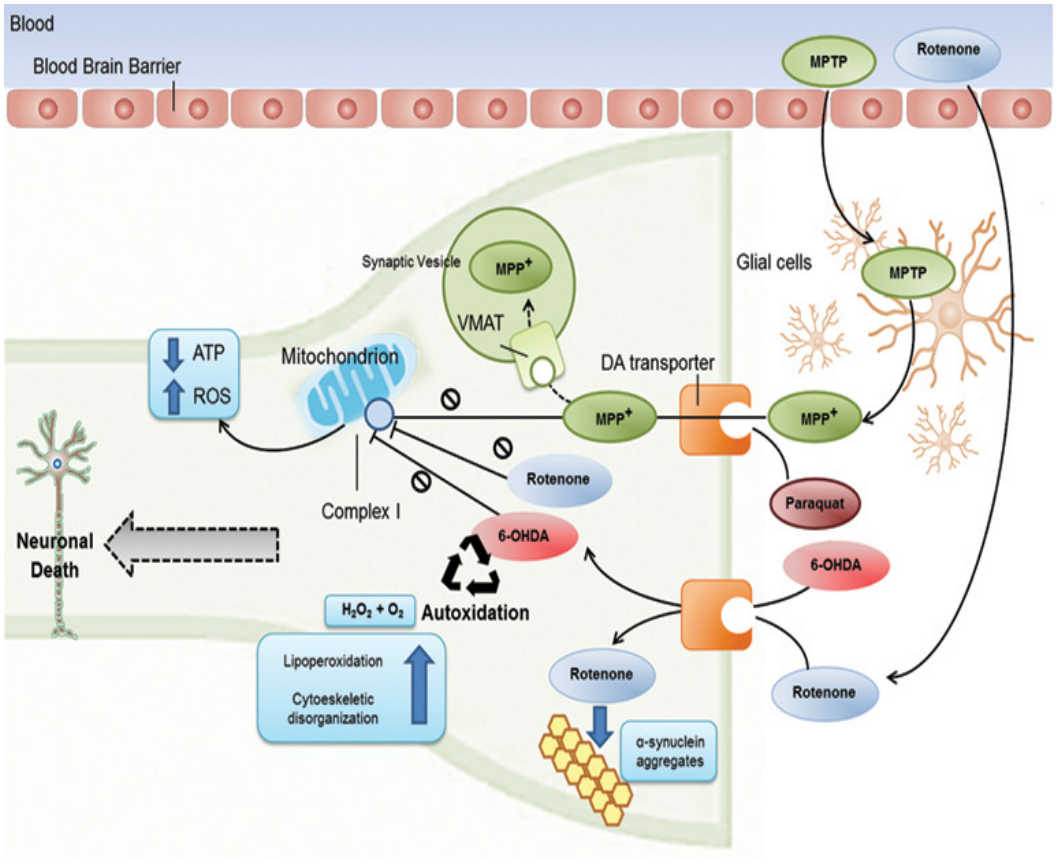


Figure 1.6: Diagrammatic representation of toxin induced model of PD (Source: Blesa *et al.*, 2016)

1.7. Limitations of toxin-induced rodent models

Rodent models are very helpful for the study purpose as they produce histological, biochemical, pathogenic and clinical features of sporadic PD. Rodent models have various advantages as well as few disadvantages. For instance, the 6-OHDA models do not show the cytoplasmic inclusion, Lewy body that is seen in the PD patients. Due to lack of their presence in the environment, humans do not directly expose with it. Therefore it is very difficult to extrapolate the outcomes of this model to human **(Betarbet *et al*., 2002; Yadav *et al.*, 2012a)**. The MPTP model is acute and not very successful to mimic all the features of PD. MPTP model also fails to produce Lewy body and also it is non-progressive and reversible. However, rotenone is supposed to be a good model as it mimics almost all the features of sporadic PD like Lewy body formation, dopaminergic neurodegeneration, mitochondrial complex I inhibition and free radical formation. But owing to its non-specific nature it can also degenerate the other non-dopaminergic neurons, this model was not very much used **(Yadav *et al*., 2012a)**. The biochemical and pathological feature of PD is also produced by combined maneb and paraquat model but the exact mechanism of neurodegeneration is yet not known. Therefore it is required to look for the reliable and accurate model for the further extrapolation of the study.

1.8. Genetic models

In order to develop more understanding about the molecular mechanism of PD pathogenesis, the genetic models have been developed by mutations in the LRRK2 and α-synuclein that may lead to autosomal dominant and DJ-1, PINK1 and parkin mutations may cause the autosomal recessive PD **(Dawson *et al*., 2010)**. The genetic form of PD may also induce due to the deficiency of transcription factor such as,

Pitx3, synphilin1, Nurr1 and nuclear receptor subfamily 4 groups A member 2. To figure out the PD mechanism, α-synuclein aggregation, overexpression and its clearance may also be understood well by such mutations in the genes (**Le *et al*., 2003; Bekris *et al*., 2010**).

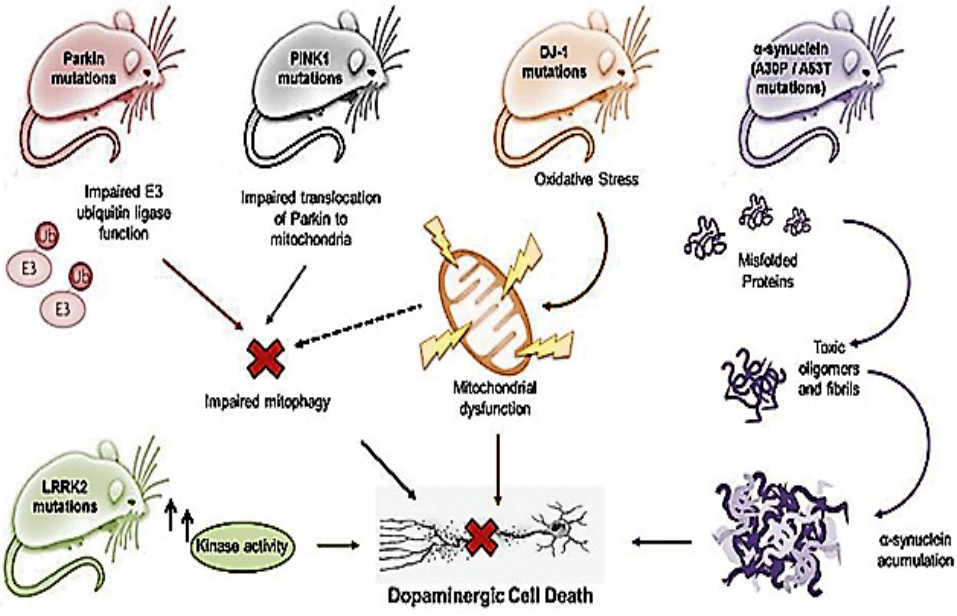


Figure 1.7: Diagrammatic representation of genetic mutations in PD (Source: Blesa et al., 2016)

1.9. Limitations of genetic models

Lack of extensive and progressive dopaminergic neurodegeneration is the major drawback of the mutations of the PD related genes in the rodent models. Study of early onset of the disease could be done with the help of these models (**Chesselet *et al*., 2008**). In humans, the mutations in multiple genes and combination of mutation in one gene with environmental insults may also responsible for PD. Therefore all the pathologies of PD may not produce the model that has the single gene mutation (**Duty and Jenner, 2011**).

1.10. Newer animal models

The pesticides used regularly in our day-to-day life are harmful and are toxic to the nervous system of mammals. Thus these may also cause the neurodegeneration and responsible for the onset of PD. In order to understand the mechanism of these toxins, many rodent models have been developed but no one was perfectly able to mimic all the features of PD. Thus in the current scenario, there is a strong requirement of a model that can mimic the sporadic PD. Organochlorines and pyrethroids are the commonly used pesticides worldwide these days. Intense researches are going on to find out the mechanism of these pesticides. Organochlorine pesticide and dieldrin were found to induce the death of dopaminergic neurons in the postmortem brain of PD patients (**Uversky *et al*., 2001; Kanthasamy *et al*., 2005**). Pyrethroids such as cypermethrin, deltamethrin and permethrin are also found to toxic to the nervous system and contribute as a risk factor for PD (**Tayebati *et al*., 2009; Dodd and Klein, 2009; Elwan *et al*., 2006; Xiong *et al*., 2016**).

1.11. Cypermethrin

Cypermethrin (RS)-α-cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate, is chemically modified from natural pyrethrin, derived from chrysanthemum plant. Cypermethrin is a class II pyrethroid insecticide and after the chemical modification, its efficacy and stability in the environment increase. It is generally used to control many pests including moths pests of cotton, fruits and vegetables in agricultural fields. Cypermethrin easily crosses the blood brain barrier owing to its lipophilic property and causes the neurotoxicity in the central nervous system and induces the behavioral deficits

(Manna *et al*., 2005; Wolansky and Harill, 2008). Pyrethroids such as cypermethrin cause the hyper-excitation and hyperpolarization by extending the opening of sodium ion channels in the central nervous system **(Crawford *et al*., 1981; Kirby *et al*., 1999; Eells and Dubocovich, 1988; Narahashi *et al*., 1992)**. Additionally, Cypermethrin modulates the level of gamma-aminobutyric acid (GABA) **(Gilbert *et al*., 1989; Manna *et al.*, 2005; Staatz *et al.*, 1982)**. Furthermore, cypermethrin induces neurotoxicity by means of generating free radicals **(Giray *et al*., 2001; Kale *et al*., 1999; Sian *et al*., 1994)**. By inducing the oxidative stress, that is critically responsible for the loss of dopaminergic neurons in the substantia nigra, cypermethrin could be regarded as an appropriate pesticide and may be implicated in PD pathogenesis **(Nasuti *et al*., 2007; Singh *et al*., 2010; Singh *et al*., 2011a)**. Cypermethrin shows more pronounced effects when exposed developmentally and re-exposed upon adulthood **(Richardson *et al*., 2006; Thiruchelvam *et al*., 2000)**.

1.12. Effect of cypermethrin exposure on dopaminergic and non-dopaminergic neurons

Cypermethrin affects the dopaminergic neurons in the nigrostriatal region of the brain in a dose dependent manner. Its accumulation in the brain in a considerable amount induces the oxidative stress and thus results in the neurotoxicity. The cypermethrin induced model mimics the symptomatic features of PD **(Singh *et al*., 2012a; Nasuti *et al.*, 2007)**. In addition to the dopaminergic neurons, cypermethrin also affects the non-dopaminergic neurons such as GABAnergic neurons of the brain **(Manna *et al*. 2005)**. The pyrethroid exposure also causes the decrease in the level of serotonin and its metabolites **(Larranaga *et al*., 2003)**. The acetyl and butyryl

cholinergic neurons and cholinesterase enzyme are also affected by cypermethrin dose dependently **(Anwar, 2003)**.

1.13. Mechanisms of neurotoxicity in PD

1.13.1. Apoptosis

Distinct morphological features and energy dependent biochemical mechanisms are generally involved in the process of programmed cell death or apoptosis. Inappropriate apoptosis could be a reason for the onset of various disorders including neurodegenerative diseases. Apoptosis generally takes place as a homeostatic mechanism in order to maintain the cell population **(Elmore *et al*., 2007)**. Programmed cell death is the characteristic feature of multicellular organisms. Enhanced level of apoptosis could lead to the death of cells that may or may not be required anymore for normal function **(Vila *et al*., 2003)**. Elevated number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive neurons in the PD brain focused on the role of apoptosis **(Mochizuki *et al*., 1996)**. In the PD patients brain the elevated level of anti-apoptotic protein B-cell lymphoma extra large (Bcl-xL), Bax and caspase-3 are reported to be present in greater extent and redistributed in the subcellular regions **(Hartman *et al*., 2001; Tatton, 2000)**. Other apoptosis related proteins such as caspase-9 and caspase-8 are also getting activated in the brain of PD patients **(Viswanath *et al*., 2001)**. The role of apoptosis have been also shown in the toxin-induced rodent models **(Chesselet and Richter *et al*., 2011; Bernstein *et al*., 2011; Singhal *et al*., 2011; Yadav *et al*., 2012a; Favaloro *et al*., 2012)**. Cypermethrin is also known to induce the oxidative stress and apoptosis **(Tiwari *et al*., 2010; Tiwari *et al*. 2012)**. The onset of mitochondria

mediated intrinsic apoptosis is reported in cypermethrin-induced Parkinsonism through increased oxidative stress.

1.13.2. Mitochondrial dysfunction

Mitochondrion is known as the energy centre of the cell and its electron transport chain contains several redox centres and these are the primary source for the generation of free radicals. These free radicals in response lead to the enhanced level of oxidative stress and death of neurons **(Subramaniam and Chesselet, 2013; Szarka *et al*., 2014; Dai *et al*., 2014)**. MPTP, maneb, paraquat and rotenone are the environmentally present toxins and interfere with the mitochondrial complex I/III. Mitochondrial complex inhibition leads to the production of robust amount of reactive oxygen species and damage to the lipid, proteins and DNA **(Yadav *et al*., 2012a; Dai *et al*., 2014)**. Besides respiratory chain inhibition, mitochondrial dysfunction may also take place due to the cellular oxidative stress, mitochondrial genes mutations, mitochondrial DNA deletions and changes in the mitochondrial morphology **(Subramaniam and Chesselet, 2013)**.

1.13.3. Oxidative stress

The free radical generation and reduced anti-oxidant defence system in neuronal system is induced upon systemic exposure of cypermethrin **(Giray *et al*., 2001)**. The catalytic activities of anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase are modulated under the influence of cypermethrin **(Jin *et al*., 2011)**. Additionally, the expression and catalytic activity of xenobiotic metabolizing enzymes also get altered upon cypermethrin exposure **(Giray *et al*., 2001; Floodstrom *et al*., 1988)**.

1.13.4. DNA damage

The DNA damage is induced by cypermethrin at a low concentration in human lymphocytes and basal ganglion **(Kocaman and Topaktas, 2009)**. Inside the brain cypermethrin induces damage to DNA and reduces the proliferation of cell. Cypermethrin exposure also results in the germline mutations and teratological deformities **(Batiste-Alentorn *et al*., 1986; Anwar, 2003; Bhunya and Pati, 1988)**.

1.13.5. Alteration in ion channel activity

Cypermethrin primarily prolongs the opening of ion channels of insects and mammals due to the phylogenetic similarity in the neuronal system. The functions of sodium, potassium, calcium and chloride channels get modulated under the influence of cypermethrin. Furthermore, not only ion channels but the activity of receptors like, GABA, glutamate, acetylcholine and ATPases are also modulated by cypermethrin. In the similar fashion as other pyrethroid insecticides, cypermethrin modulated the function of the sodium ion channel by altering the potential required for their proper opening and closing **(Narahashi, 1996)**. The signal transduction machinery gets disturbed due to the hindered voltage gated calcium channels (VGCC) under the influence of cypermethrin. Potassium channel also gets affected due to the cypermethrin that is responsible for the loss of neuronal excitability and thus leading to the neurotoxicity **(Murakoshi and Trimmer, 1999; Rao and Rao, 1997)**. Chloride ion channel are regulated by GABA, one of the most prevailing neurotransmitter and cypermethrin exposure reduces the GABA mediated uptake of chloride ion and hyper-excitability leads to neurotoxicity **(Bloomquist and Soderlund, 1985; Ray et al., 1997; Ullah et al., 2006)**.

1.14. Neuroinflammation in PD

In the host cell, inflammation is the first line of defence against injury but excessive level of inflammatory response can be fatal. Neuroinflammation is the key player of Parkinson's disease (PD) pathogenesis **(Amor *et al*., 2010; Mosley *et al*., 2012; Glass *et al*., 2010; Tohidpour *et al*., 2017)**. Supportive data from epidemiological, animal, human and therapeutic studies suggests the existence of the neuroinflammatory cascade in the disease **(Mosley *et al*., 2006; Tansey and Goldberg, 2010)**. The involvement of inflammation in the PD was first evident from the report of James Parkinson's in the early nineteenth century on the first clinical and pathological description of the disease **(Goetz *et al*., 2011)**. In the twentieth century direct evidence was provided after the systemic post-mortem analysis of the brain of PD patients **(Rocha *et al.*, 2015)**. However the exact mechanism underlying the process of neurodegeneration in the PD is yet obscure but neuroinflammation is supposed to participate actively in this process **(Aldakheel *et al.*, 2014)**. The inflammatory mediators secreted during inflammation do not only alter the immune cells but also involved in the neurodegeneration by acting on the neurons **(Lull and Block, 2010)**. Further, after the death of neurons it activates the inflammatory mechanisms ultimately lead to the vicious cycle of inflammation and death of neurons. For the tissue homeostasis inflammatory responses are essential but only in a controlled manner otherwise can be fatal for neurons when it is chronic **(Rocha *et al*., 2015; Gao *et al*., 2003)**.

1.15. Role of microglia in PD

Over a century ago Elie Metchnikoff discovered the inflammatory cell types in the larvae of starfish capable of swallowing the foreign objects and cellular debris. Through this he postulated that these cells are essential and beneficial as well to the

host **(Kaufman *et al*., 2008)**. The frontline defence of the multi-cellular organisms against infection is the inflammation. A number of human diseases including neurodegenerative diseases are closely linked to the inflammatory responses **(Rocha *et al*., 2015)**. The activated microglia was observed by McGeer and colleagues in the post-mortem substantia nigra pars compacta (SNpc) of the PD patients and first time evident the role of inflammation in PD. Many studies have proved that microglial activation and subsequent neuroinflammation plays a role in the PD pathogenesis. The chronic activation of microglia leads to the death of neurons in PD **(Tansey and Goldberg, 2010; Wang *et al*., 2015)**. The microglial activation may take place either directly through the toxic insult, invading pathogens or endogenous protein or indirectly via dying neurons. The repetitive cycle of microglial activation in response to the death of neurons is referred to as reactive microgliosis and is responsible for the many brain pathogenesis **(Collins *et al*., 2012)**.

The substantia nigra is more susceptible for the microglia-mediated neurotoxicity due to the presence of higher number of microglia and these are responsible for the death of dopaminergic neurons **(Bartels *et al*., 2010; Frank-Cannon *et al*., 2009)**. Hence the elevated level of microglial activation and subsequent neuroinflammation may be potentially magnifying the neuronal damage in the PD. Many substances that are produced by the neuronal death and can stimulate microglial activation are α-synuclein-aggregates, neuromelanin, adenosine triphosphate (ATP) and matrix metalloproteinase-3 (MMP-3) **(Kraft and Harry, 2011)**. In PD the major constituents of Lewy bodies are the aggregated α-synuclein and are surrounded by the activated microglia or inflammatory mediators **(Glass *et al*., 2010)**. The phagocytosis of extracellular aggregates of the α-synuclein and NADPH oxidase

activation is essential for the subsequent microglial activation and the dopaminergic neurodegeneration (**Lull and Block, 2010**). The stressed DA neurons release a neuro-pigment, neuromelanin which induce the activation of microglia though the proteasomal inhibition (**Collins *et al*., 2012**).

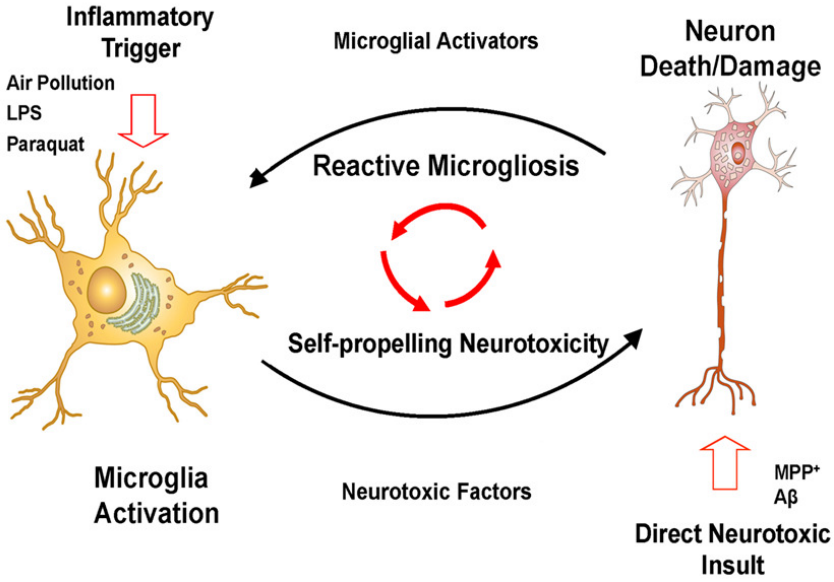


Figure 1.8: Diagrammatic representation of neuroinflammation in neuronal death
(Source: Block *et al.*, 2007, Nature reviews neuroscience)

1.16. Neuroinflammation and PD associated genes

According to earlier studies few genes related to PD and also responsible to regulate the microglial immune responses in the brain. Among those, all α-synuclein is a gene after missense mutations responsible for the familial form of PD (**Polymeropoulos *et al*., 1997**). The abnormal accumulation of α-synuclein is responsible for the pathological hallmark of the disease. The variations present in the LRRK2 is responsible for the familial and sporadic form of PD (**Paisan-Ruiz *et al*., 2004; Zimprich *et al*., 2004; Simon-Sanchez *et al*., 2009; Satake *et al*., 2009**). It is known that LRRK2 is responsible for the mutations in the periphery. E3-ubiquitin ligase is

another PD associated gene known as parkin. Mutation in parkin is responsible for the recessive inheritance of PD **(Kitada *et al*., 1988)**. PINK 1 also regulates the activity of NF-κB. The DJ-1 expression is known to occur in the reactive microglia of PD patient's brain with increased expression.

1.17. Treatment strategies for PD

Data from earlier works have been suggested that upon disease onset, significant loss of dopaminergic terminals takes place. Thus there is a huge opportunity to protect the remaining essentials of neurons **(Burke *et al*., 2013)**. Earlier data also indicates that in both the conditions either at the onset or at disease terminus the loss of substantia nigra neurons was compensated by the striatal dopaminergic terminal loss. Therefore during the course of disease the loss of neuronal cell bodies is limited as compared to the terminal loss and that may progressively leads to the clinical worsening and ultimately appears in the form of motor dysfunctions **(Burke *et al*., 2013)**. The mechanism that may leads to the terminal loss is yet not clear but inflammatory agents like, COX-2 are known to present in terminals and are responsible for inflammation **(Bartels *et al*., 2010)**. Thus the anti-inflammatory drugs could be beneficial in order to overcome from the loss of dopaminergic terminals.

1.17.1. Anti-inflammatory drugs and PD

Many studies have suggested the importance of neuroinflammation in the initiation and progression of PD. Thus it is necessary to develop such a technique that can intervene the inflammatory pathway induced by the microgliosis. Many compounds that have the anti-inflammatory property showed the potential neuroprotection for dopaminergic neurons against various PD models **(Bartels *et al*., 2010)**. Toxin

induced models like 6-OHDA, LPS showed the significant loss of dopaminergic neurons. Several anti-inflammatory drugs are known to reduce the microgliosis. Among these, the non-steroidal anti-inflammatory drugs are known to reduce the dopaminergic neurodegeneration and microglial activation (**Matteo *et al*., 2006; Mohanakumar *et al*., 2000; Sanchez-Pernaute *et al*., 2004; Gao *et al*., 2011a**).

1.17.2. Nonsteroidal anti-inflammatory drugs (NSAIDs) in relation with PD

The key role of neuroinflammation in the PD has been supported by several epidemiological studies (**Rocha *et al*., 2015**). Drugs having the rescuing capacity for the dopaminergic neurons from microgliosis and inflammation may contribute in the amelioration of Parkinsonian symptoms by delaying the onset and halting or slowing the progression of PD. Many drugs are known so far for their capacity to inhibit the microgliosis or neurotoxicity (**Tansey and Goldberg, 2010**). Since many experimental and clinical studies have proved that NSAIDs may use as a therapeutic agent for the treatment of PD thus much attention have been drawn on it (**Esposito *et al*., 2007**). Many epidemiological studies have investigated the link between the regular use of NSAIDs and the risk of PD. Epidemiological studies strongly support the role of conventional NSAID, ibuprofen, in the risk reduction of the PD development (**Chen *et al*., 2003; Chen *et al*., 2005**).

1.17.3. Ibuprofen

Ibuprofen is a commonly used non-steroidal anti-inflammatory drug possess potent anti-inflammatory anti-PD efficacy (**Asanuma *et al*., 2006; Casper *et al*., 2000**). Ibuprofen is known to inhibit the cyclooxygenase-2 expression and neurodegeneration of dopaminergic neurons in the toxin-induced animal models of

PD **(Hsieh *et al*., 2011)**. Ibuprofen is preferred over other NSAIDs because of so many reasons. Earlier reports have suggested that ibuprofen but not other NSAIDs provide protection against oxidative stress and inflammation-induced Parkinsonism **(Tsuji *et al*., 2009; Casper *et al*., 2000)**. Ibuprofen is known to regulate some pathways and expression of few mediators, which are regulated by various anti-PD drugs, i.e., expression of the peroxisome proliferator activated receptor-γ, dopamine positive transporter signals and activation of microglial cells, which are not known to be regulated by other NSAIDs **(Tsuji *et al*., 2009)**. Without producing any considerable side-effects ibuprofen is known to enhance the loss of dopamine (DA) level **(Kurkowska-jastrzebska *et al*., 2006)**. Because of its cost effectiveness, easy availability, common practice and the nice public perception it may be selected over other NSAIDs to treat PD. If ibuprofen minimizes the severity of Parkinsonism in rodents by providing protection from Parkinsonian features, it could be an asset to PD research since having relatively less toxicity as compared to many other anti-PD drugs.

CHAPTER 2

AIMS AND OBJECTIVES

Chapter 2

Aims and objectives

Inflammation plays an important role in the PD pathogenesis. The most severely affected system in PD is the nigrostriatal dopamine pathway, which starts from substantia nigra and terminates in the striatum. The main neuropathological manifestation of the disease is the dysregulation of dopaminergic neurons of the nigrostriatal region (**Arimoto *et al*. 2007**). While the exact cause of dopaminergic neuronal death in PD is yet elusive. Role of inflammation is supported by the reports, which have shown the increased number of the reactive microglia in the substantia nigra. Experimental and epidemiological studies have revealed that the ibuprofen could be beneficial for providing protection from the loss of dopaminergic neurons. The present study was therefore undertaken to decipher the role of ibuprofen against cypermethrin induced changes in the striatal dendrites and spines and dopaminergic neurons. The major objectives of the study have been mentioned below:

(1) To investigate the salvaging potential of ibuprofen against cypermethrin-induced nigral mitochondrial dysfunction and dopaminergic neuronal loss associated with Parkinsonism

(2) To examine the consequence of ibuprofen exposure on cypermethrin-induced changes in dendritic arborization, ramification and spine density of the striatal neurons

(3) To investigate the crosstalk of ibuprofen-mediated alleviation in the cypermethrin-induced changes in nigral mitochondrial function and striatal dendritic arborization, ramification and spine density with anti-neuroinflammatory potential to delineate the mechanism of neuroprotection

CHAPTER 3

Protective effect of ibuprofen against cypermethrin-induced mitochondrial dysfunction and dopaminergic neurodegeneration

CHAPTER 3

Protective effect of ibuprofen against cypermethrin-induced mitochondrial dysfunction and dopaminergic neurodegeneration

3.1. Introduction

Neuroinflammation plays an important role in the progression of the PD. It is the most common chronic devastating movement disorder, characterized by the depleted level of dopamine in the striatum. Epidemiological and experimental studies have shown that PD is resulted due to the prolonged exposure of environmental toxins on the genetically prone aged animals and humans **(Uversky, 2004, Cicchetti *et al*., 2009)**. The environmental theory of PD has got impetus after a discovery that MPTP, a toxin present in the synthetic heroine, lead to the development of PD like characteristic meperidine addicts **(Uversky, 2004, Yadav *et al.*, 2012a)**. Afterwards, several environmental toxins have been found to be toxic to the neuronal cells. In this perspective, cypermethrin, a pyrethroid pesticide, is also found to induce the nigrostriatal dopaminergic neurodegeneration **(Singh *et al*., 2011b; Singh *et al*., 2012a)**. Farmers and industrial workers in their day-to-day life are exposed to cypermethrin since it is produced in pesticide industry and to control insects in agricultural and household sectors **(Crawford *et al*., 1981)**. Cypermethrin does not elicit significant toxicity on dopaminergic neurons at short term exposure. However, its long term exposures and moderate doses induce harmful effects on the striatal

dopamine depletion, nigral TH-immunopositive neuronal loss, microgliosis and motor impairments **(Singh *et al*., 2016; Agrawal *et al*., 2015b; Singh *et al*., 2012b)**. Microgliosis is a basic characteristic of PD in animals and humans. Epidemiological and animal experimentation have supported the role of neuroinflammation in PD **(Mosley *et al*., 2006)**. While extensive studies have been performed on the etiology of PD, little information is available about the mechanism of PD pathogenesis **(Bartels *et al*., 2010)**. Mitochondrial dysfunction, chronic inflammation and oxidative stress have been found to play synergistic roles in PD **(Pinto *et al*., 2016; Hunter *et al*., 2007)**. Mitochondrial dysfunction enhances the oxidative stress and hampers the energy metabolism leading to the disturbance in the normal physiological function **(Glinka and Youdim, 1995; Glinka *et al*., 1996; Yadav *et al*., 2012a; Dixit *et al*., 2013)**. Level of the mitochondrial complex I is found to be significantly reduced in the substantia nigra of the postmortem brain of PD patients **(Schapira *et al.*, 1990)**.

Neuroinflammatory processes are known to elevate the level of prostaglandin and cyclooxygenase (COX). Neuroinflammation has been found to be initial in the neurodegenerative disease, like PD **(Minghetti, 2004)**. An interaction of the COX-2 with apoptotic neurons is found to promote COX-2 expression and prostaglandin synthesis. The microgliosis and underlying inflammatory responses are known to induce the deleterious effects which could be amplified by COX-2 **(Bartels *et al.*, 2010)**. The activated microglia is present in higher quantity in the substantia nigra of the brain and known to involve in the process of neurodegeneration. Microgliosis occurs at the early onset of the disease and progresses to the lengthened fibers like dendrites and indicates the involvement of inflammation in PD **(Bartels *et al*., 2010, Sugama *et al*., 2003; Asanuma *et al*., 2007)**.

Due to the lack of the permanent treatment of PD, many natural and synthetic agents have been tested to check their neuroprotective potential. Ibuprofen, a nonsteroidal anti-inflammatory drug, is found to be effective against PD in many experimental studies **(Manthripragada *et al.*, 2011)**. Ibuprofen is known to inhibit the cyclooxygenase-2 (COX-2) activity and protects the loss of dopaminergic neurons even in a few toxin models of PD **(Singh *et al.*, 2016)**. Although the studies have shown that ibuprofen exposed animals are less susceptible to developing PD, the mechanism underlying the process of neuroprotection is yet not clear. In the present study has been made to understand the mechanism by which ibuprofen offers protection against cypermethrin induced Parkinsonism.

3.2. Materials and methods

3.2.1. Chemicals

Anti-mouse/rabbit biotinylated secondary antibodies, cypermethrin, ibuprofen, were purchased from Sigma Aldrich, St. Louis, MO, USA. Goat serum was purchased from GeNei Bangalore, India. Copper sulfate, diethyl ether, sodium dodecyl sulfate and sodium potassium tartrate were obtained from Merck India Private Limited, Mumbai, India. Neg-50 from Richard Allen Scientific (Kalamazoo, MI, USA). Primary antibodies against tyrosine hydroxylase (TH) and integrin-αM were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Copper (II) sulfate 5-hydrate, methanol, potassium chloride, potassium dihydrogen orthophosphate, sodium carbonate and sodium thiosulfate were purchased from Merck Biosciences (Darmstadt, Germany). Formic acid was obtained from Sisco Research Laboratories Private Limited (Mumbai, India). Remaining other chemicals were purchased locally.

3.2.2. Animal treatment

The study was conducted after the approval of the institutional ethics committee for the use of laboratory animals. Cypermethrin (1.5 mg/kg; twice a week) was intraperitoneally (i.p.) given to the male pups during its postnatal days along with respective vehicles. After two months the male rats were re-challenged with cypermethrin (15 mg/kg; twice a week) up to 12 weeks along with their respective controls **(Singh *et al*., 2012b)**. The subsets of adult rats were treated with ibuprofen (20 mg/kg, i.p. daily) along with respective controls **(Cao *et al.*, 2012)**. 24 h later animals were sacrificed by cervical dislocation upon completion of cypermethrin treatment. The brain was either used immediately or stored at -80^0 C for further experimental purposes.

3.2.3. Measurement of dopamine level

Striatal dopamine was measured using the standard protocol described elsewhere **(Tareke *et al.*, 2007)** by using liquid chromatography-mass spectrometry. Ultra-performance liquid chromatography (UPLC) (Acquity-Waters, Milford USA) was coupled with API-4000 mass spectrometer detector (AB ScieX) and electrospray ionization device. Striatum was dissected out of the brain and homogenized, sonicated and centrifuged at 15,000 xg for 10 min at 4^0 C. Supernatant was filtered through a syringe filter (0.22 µm) and a measured volume of 7.5 µl was injected into an auto-sampler coupled with UPLC. Supelco Ascentis Express C-8 column (50 mm x 2.1 mm, 1.7 µm particle size) was used for the separation of the analyte. Isocratic elution of mobile phase began with the 100%-0.1% formic acid at a constant flow of 0.25 ML/min. The content of dopamine was calculated in µg/gram of protein and is represented as percent of saline control (saline).

3.2.4. Cryosectioning

The cryosectioning was performed according to the protocol described elsewhere **(Singh *et al.*, 2012 b)**. In brief, after completion of treatment schedule ether was used to anesthetize the animals. The perfusion was performed intracardially with saline and 4% paraformaldehyde in phosphate buffered saline (PBS) at a constant flow rate of 20 ml/min for each 4 min. The paraformaldehyde solution (10% w/v) was used to post fix the brain minimum for a day after dissection. Sucrose solution was prepared in variable concentrations (10%, 20% and 30% w/v each) in PBS and brain was serially cryoprotected in it. The coronal sections (20 µm) of the brain were cutted using cryostat and ice cold PBS was used for their collection. The processing of sections was performed immediately or stored at 4^0C until used.

3.2.5. Neuronal nuclei (NeuN)/tyrosine hydroxylase (TH)-immunoreactivity and integrin-αM-immunoreactivity

The NeuN/TH-immunoreactivity, counting of neurons and integrin-αM-immunoreactivity was performed in the substantia nigra, according to the previous protocol **(Singh *et al*., 2012 b, Srivastava *et al*., 2012)**. A similar protocol was used to perform TH-immunoreactivity in the striatum **(Singh *et al*., 2011a)**. In brief, nigral/striatal sections were washed in PBS and further the sections were incubated for 15 min in the solution of hydrogen peroxide (0.5% v/v) and methanol (40 % v/v) in the PBS for performing the endogenous peroxidase activity. The sections were incubated in blocking solution (2% normal goat serum, 0.1% triton x-100 and 1 % bovine serum albumin (BSA) for 2 h after washing with PBS. Again the sections were washed with PBS and further incubated with a cocktail of monoclonal anti-NeuN (1:500) and anti-TH (1:500) primary antibodies (only TH antibody used for the striatum) for performing NeuN/TH-immunoreactivity and anti-integrin-αM

(1:500) antibody for microglial activation for 48 hours at 4^0C. Subsequent washing with PBS was performed sections were incubated in anti-mouse as a secondary antibody for NeuN/TH-immunoreactivity while anti-rabbit was used for the microglial activation (1:1000) for 2 h followed by streptavidin (1:1000) for 1 h. Finally, the sections were rinsed with PBS and developed with 3, 3'-diaminobenzidine. Dehydration of sections was performed using graded ethanol (25%, 50%, 75% and 100%) and on glass slides, mounting was done with dibutyl phthalate xylene. The visualization and imaging of sections were performed using the light microscope. In serial sections, the counting of cells was performed with the help of the Leica QWin image analysis software (Leica Microsystems, Heerbrugg, Switzerland).

3.2.6. Mitochondrial fraction isolation

Using differential centrifugation method mitochondrial fraction was fractionated from the nigrostriatal tissues of rat brain **(Dixit *et al.*, 2013)**. The homogenization of tissue was performed in mitochondria isolation buffer having 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (20 mM; pH 7.2), sucrose (75 mM), mannitol (215 mM), ethylene glycol tetraacetic acid (1 mM), ethylenediaminetetraacetic acid (EDTA; 1mM), phenylmethanesulfonylfluoride (1 mM) and protease inhibitor cocktail. Centrifugation of homogenate was performed at 1000 ×g for 5 min at 4^0C and the supernatant was collected. The same buffer was used for the re-suspension of pellet, centrifugation was done at 1000 ×g for 5 min at 4^0C and the supernatant was collected. After pooling of both the supernatant the centrifugation was performed at 13,000 ×g for 10 min at 4^0C. The pellet was washed properly two times and suspended in a minimal amount of mitochondrial isolation buffer. The suspension

used as the mitochondrial fraction after performing the freezing, thawing and sonication.

3.2.7. Mitochondrial complex I activity measurement

Mitochondrial complex I activity was measured according to the previous protocol using spectrophotometric method (SpectraMax M5, Molecular Devices, CA, USA) **(Dixit *et al.*, 2013)**. In an assay buffer (pH 7.4) containing potassium phosphate (35 mM), sodium cyanide (2.65 mM), magnesium chloride (5 mM), EDTA (1 mM), fatty acid free BSA (1 mg/ml) and antimycin (2 µg/ml), mitochondrial fraction isolated from the rat brain and ubiquinone 1 (0.05 mM) was added. For the initiation of the reaction, after 2 min of incubation, the reaction mixture was added with nicotinamide adenine dinucleotide (NADH; 5mM). At 340 nm for 3 min of the interval of 15 sec, a decrease in the absorbance was noted. The activity of mitochondrial complex I was expressed as nanomoles of NADH oxidized/min/mg protein.

3.2.8. Protein estimation

The protein content of mitochondrial fraction was estimated using the method of Lowry and BSA was taken as a standard **(Lowry *et al*., 1951)**.

3.2.9. Statistical analysis

Three to five independent sets of experiments consisting of an animal per group per set was used (n=5 animals per group per set). One way analyses of variance were done for comparisons between multiple groups. The data were analyzed as means ± standard error of means. The differences were considered statistically significant when ‘p’ value was less than 0.05.

3.3. Results

3.3.1. Level of dopamine

The level of striatal dopamine was found to decrease up to the significant level in cypermethrin treated rats in comparison with the saline treated animals (Figure 3.1). Ibuprofen treatment enhances the level of dopamine towards control.

3.3.2. Neuronal nuclei (NeuN) / tyrosine hydroxylase (TH)-immunoreactivity

The number of NeuN/TH-immunoreactivity and counting of cells in the substantia nigra and similarly the TH-immunoreactivity in the striatum was decreased upon the cypermethrin treatment in rats in comparison with the control (saline treated) animals (Figure 3.2a, b, 3.3a and b). The animals showed alleviation in the NeuN/TH-immunoreactivity and a number of NeuN/TH positive cells in the substantia nigra as well as TH-immunoreactivity in the striatum upon ibuprofen treatment.

3.3.3. Microgliosis

The process of microgliosis was found decreased up to a significant level upon the treatment of anti-inflammatory agent, ibuprofen. Cypermethrin treated animals showed an enhanced number of activated microglia (Figure 3.4a, b).

3.3.4. Mitochondrial complex I activity

Mitochondrial complex I activity was halted upon cypermethrin treatment in rats in comparison with the saline control animals. Ibuprofen treatment enhanced the activity of mitochondrial complex I (Figure 3.5).

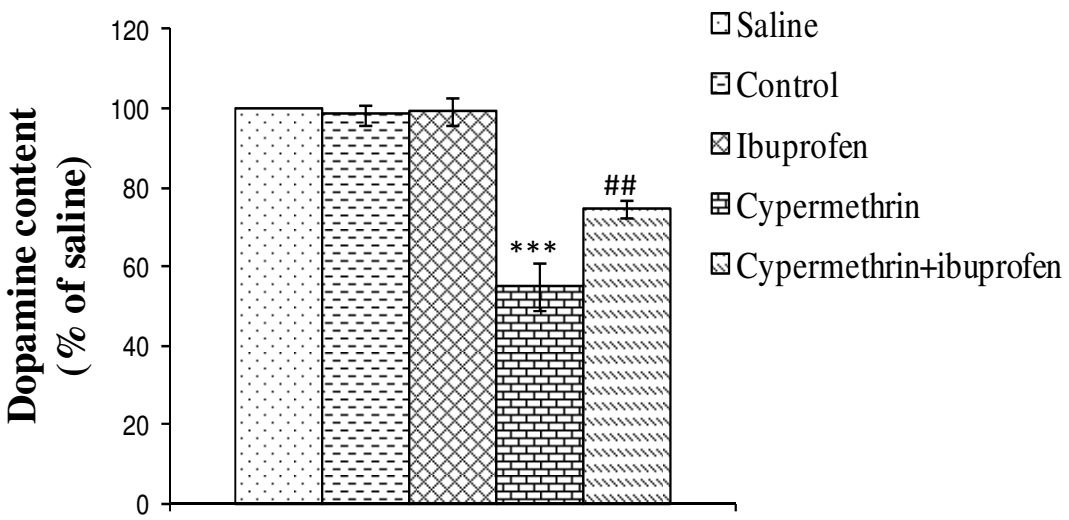
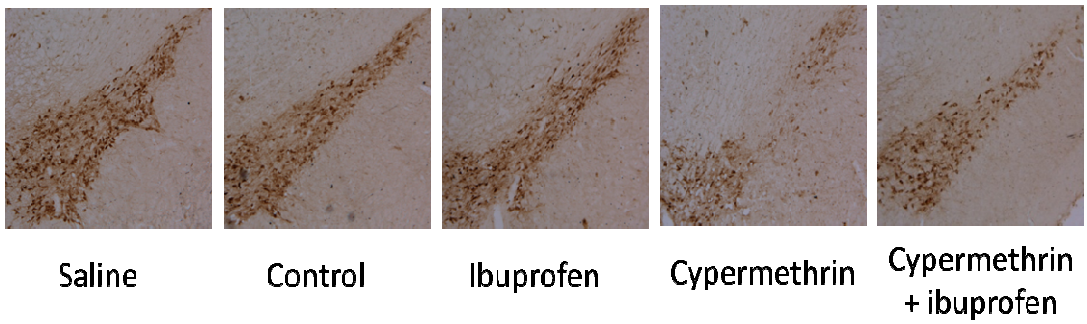


Figure 3.1: Effect of cypermethrin on the striatal dopamine content as measured by LC-MS in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated rats.

(a)



(b)

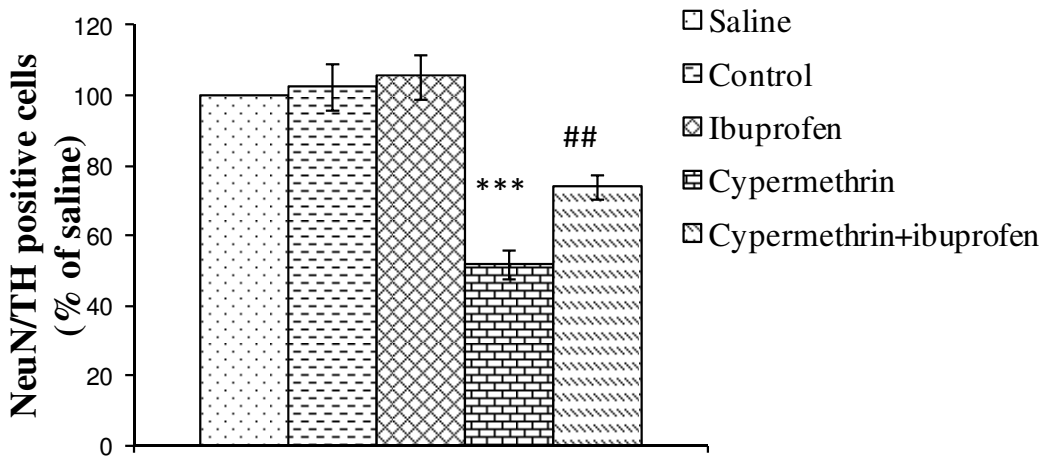
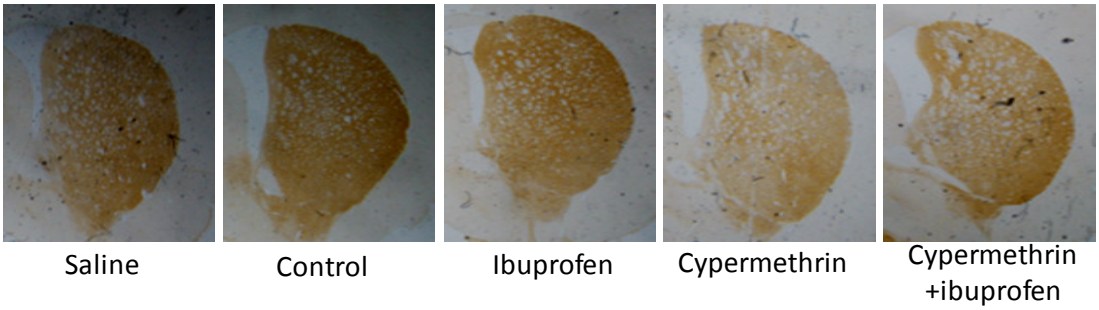


Figure 3.2: Effect of cypermethrin on NeuN/TH-immunoreactivity (a) and number of NeuN/TH positive cells (b) in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated rats.

(a)



(b)

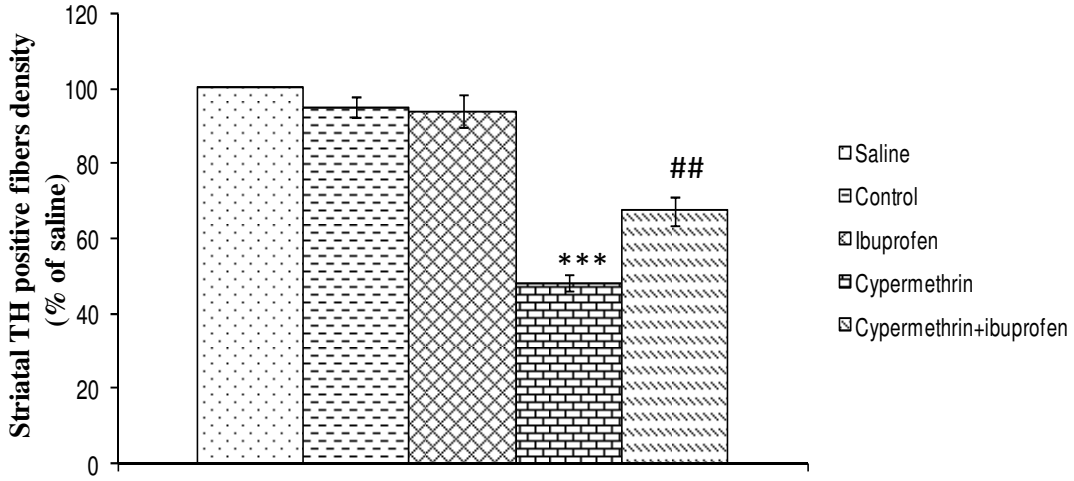


Figure 3.3: Effect of cypermethrin on TH-immunoreactivity (a) and striatal TH positive fiber's density (b) in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated rats.

Table 3.1: Effect of cypermethrin on the dopamine content in the striatum, NeuN/TH- immunoreactivity in the substantia nigra and TH-immunoreactivity in the striatum in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated animals.

Group of animals	**Dopamine content**	**NeuN-TH immunoreactivity**	**TH-immunoreactivity**
Saline	100.00±0.00	100.00±0.00	100.00±0.00
Control	98.45±2.78	102.63±6.76	94.68±2.72
Ibuprofen	99.39±3.37	105.43±6.31	93.81±4.22
Cypermethrin	54.99±5.96***	51.56±4.24***	47.89±2.26***
Cypermethrin+ibuprofen	74.80±2.21##	73.70±3.44##	67.12±3.70##

(a)

(b)

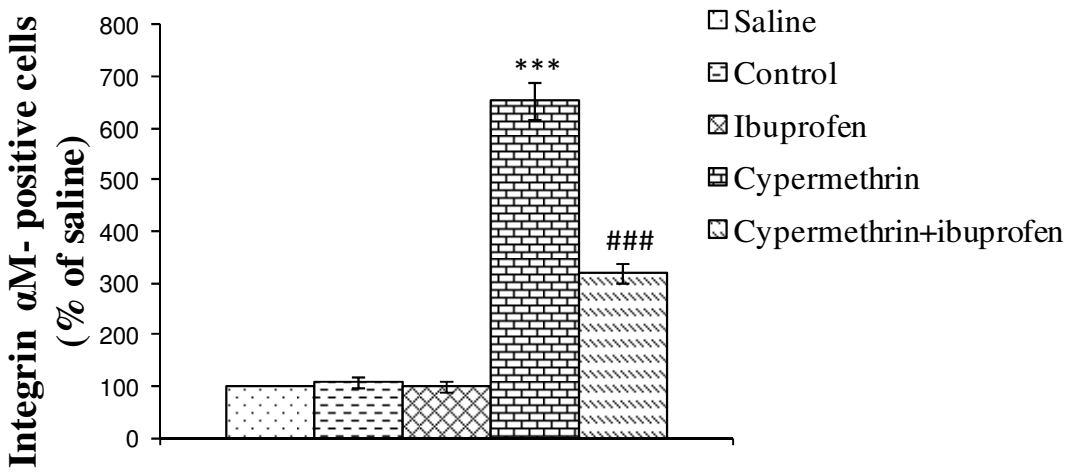


Figure 3.4: Effect of cypermethrin on microglial activation (a) and number of integrin-αM- positive cells (b) in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ### ($p < 0.001$) in comparison with cypermethrin-treated rats.

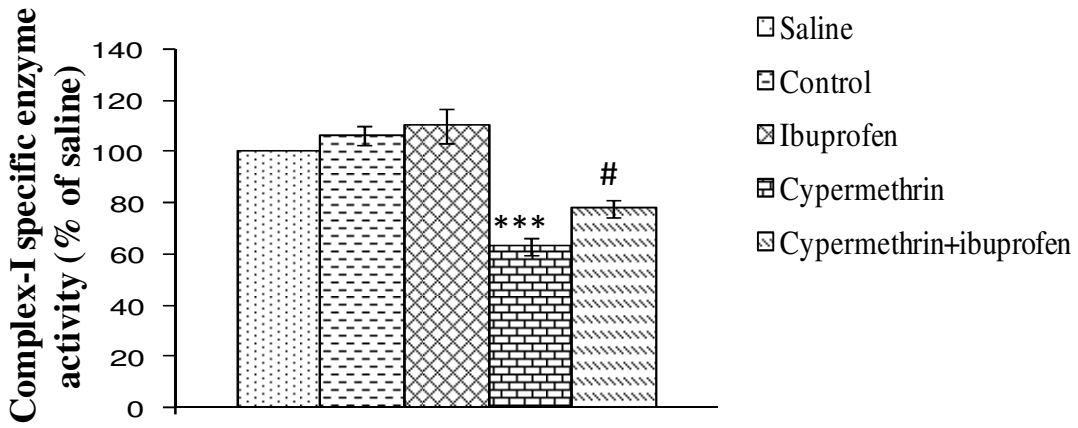


Figure 3.5: Effect of cypermethrin on mitochondrial complex I activity in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and # ($p < 0.05$) in comparison with cypermethrin-treated rats.

Table 3.2: Effect of cypermethrin on the microglial activation and mitochondrial complex I activity in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ### ($p < 0.001$), # ($p<0.05$) in comparison with cypermethrin-treated animals.

Group of animals	Integrin α-M-immunoreactivity	Mitochondrial complex-I activity
Saline	100.00±0.00	100.00±0.00
Control	107.59±10.54	106.01±3.90
Ibuprofen	99.28±9.84	109.83±6.63
Cypermethrin	653.14±36.87***	62.70±3.52***
Cypermethrin+ibuprofen	319.91±19.17###	77.59±3.39#

3.4. Discussion

Microgliosis occurs in the normal aging brain and results in the neuroinflammation, a common feature of neurodegenerative disease like PD. Treatment with ibuprofen, a nonsteroidal anti-inflammatory agent, is known to reduce the risk of PD (**Bartels *et al*., 2010**). In the present study, we have investigated the role of anti-inflammatory agent, ibuprofen, against cypermethrin induced toxicity in the dopaminergic neurons. We have tried to correlate these changes with that of dopaminergic neuron morphology that occur after toxic insult. In order to find out the role of ibuprofen against cypermethrin induced changes, we have accessed the basic Parkinsonian features like dopamine level in the striatum, number of NeuN/TH positive neurons and microgliosis.

Cypermethrin is lipophilic in nature thus easily gets inside the brain and causes the neurodegeneration. The striatum is endowed with dopamine and responsible for the co-ordination of body movement. Depleted level of dopamine causes the movement disorder like PD (**Singh *et al*., 2012b; Singh *et al*., 2016**). Since the reduction in the level of dopamine in the striatum, number of NeuN/TH positive neurons and increased number of activated microglia have been already reported against cypermethrin induced Parkinsonism in rats (**Singh *et al*., 2012b; Agrawal *et al*., 2015b**). For the validation purpose, we have performed these basic parameters of Parkinsonism by employing a different tool such as NeuN/TH-immunoreactivity and LC-MS in cypermethrin intoxicated animals in the presence or absence of ibuprofen.

In the present study the treatment of cypermethrin in rats causes the significant decrease in the level of dopamine content in the striatum, a number of NeuN/TH positive neurons and increase in microgliosis (**Singh *et al*. 2012a; Agrawal *et al*., 2015b**). The changes induced by cypermethrin were normalized after ibuprofen

treatment in rats **(Singh *et al*., 2016)**. TH- immunoreactivity was also performed in the striatum of experimental rats treated with the cypermethrin in the presence or absence of ibuprofen. The fibre density of TH positive neurons was found to be decreased in the cypermethrin intoxicated rats while ibuprofen significantly recovered the loss **(Singh *et al*., 2011a)**. As it is already reported that dopamine synthesis takes place in the substantia nigra and unloaded in the striatum **(Tripathi *et al*., 2017)**. Thus the depletion in the level of dopamine also causes the structural changes in the neurons.

Mitochondrion works as an epicenter and often considered as the power house of the cell by producing cellular energy and regulating oxidative phosphorylation. It also plays role in the metabolism of lipid, the formation of the iron sulfur cluster, maintenance of intracellular calcium level, apoptosis and cell signaling which have been found to be malfunctioning in PD **(Scheffler, 2001; Dixit *et al*., 2013)**. Various complexes that are present in the inner mitochondrial membrane are involved in the electron transport chain. The ATP production occurs through the transfer of electrons and thus generates an electrochemical gradient across the inner membrane of mitochondria **(Marchi *et al*., 2012; Narendra *et al*., 2008)**.

In order to confirm the effect of cypermethrin or ibuprofen on mitochondrial complex I is similar as reported earlier, we have performed the similar experiment. Cypermethrin is found to inhibit the mitochondrial complex I activity and ibuprofen rescue the changes **(Agrawal *et al.* 2015b)**. The present study indicates that cypermethrin causes microglial activation and hampers the mitochondrial function **(Singh *et al*., 2016)**. Activated microglia causes neuronal damage through the production of pro-inflammatory factors, reactive oxygen species and glutamate production **(Moore *et al*., 2010; Mosley *et al*., 2006)**. Oxidative stress related to the

dysfunctional mitochondria responsible for the cytochrome-c release and caspase-3 activation that are responsible for the apoptosis **(Banerjee *et al*., 2009; Agrawal *et al*., 2015b; Agrawal *et al*, 2015a)**. Our results are in accordance with the several previous studies which have shown similar changes after cypermethrin exposure in presence or absence of ibuprofen in experimental rats **(Singh *et al*. 2012; Agrawal *et al*., 2015b; Singh *et al*., 2016)**.

CHAPTER 4

The consequence of ibuprofen exposure on cypermethrin-induced changes in dendritic arborization, ramification and spine density of the striatal neurons

CHAPTER 4

The consequence of ibuprofen exposure on cypermethrin-induced changes in dendritic arborization, ramification and spine density of the striatal neurons

4.1. Introduction

Dopamine depletion is the characteristic feature of Parkinson's disease (PD). Dopamine is an important neurotransmitter that helps growth, development and maintenance of dendritic spines. Dopamine is mainly synthesized in the substantia nigra and unloaded in the striatum. The striatum is enriched with the medium spiny neurons. Striatal spine loss mainly responsible for the movement function and its deficiency causes the PD. Due to the dopamine depletion, there is marked deterioration occurs in the structure of dendrites and dendritic spines of medium spiny neurons (MSNs) **(Soderstrom *et al.*, 2010)**.

The nervous system is a complex structure and undergoes structural changes. Due to such changes there occurs the coordinated change in the pre and post synaptic components, changes in the branching pattern, dendrites and axons. Under the influence of different stimuli like injury, stress, alterations in the sensory environment the nervous system undergoes structural changes. Dendrites are present in a complex and beautiful arbor. The complexity of nervous system develops when dendrites reach to its correct partner **(Tavosanis, 2011)**. Cypermethrin causes the dopamine depletion in the striatum and could cause the change in the dendritic

morphology. Cypermethrin intoxication induces the microglial activation and inflammation but their role in the regulation of dendritic morphology, length and spine number is yet not known. Therefore it is quite necessary to find out the correlation between the cypermethrin induced inflammation and microglial activation and change in the morphology of dendrites. The loss of dendritic spines is directly dependent upon the extent of dopamine depletion.

Most of the striatal neurons are comprised of medium spiny neurons and the shaft of their dendritic spines synapse with the nigrostriatal dopaminergic axons (**Shin *et al*., 2016**). Formation of a proper synapse and its maintenance is essential for the proper brain functioning. In case of the neurodegenerative disease like PD early synaptic loss occurs while the process of neurodegeneration takes place at later stages (**Galli *et al*., 2014**). Many studies have revealed that the loss of synapse results into the axonal retraction and consequently leads to the death of neurons. Therefore it is necessary to prevent the synaptic terminals for preventing the neuronal death in the PD. In case of PD loss of synapses occurs in the striatum that results in the deficits of dopamine transmission. In the striatum among other neurons, MSNs represents approximately 95% population. In the striatum, the MSNs receive dopaminergic inputs from the substantia nigra. Dopamine depletion in the striatum causes the decrease in the dendritic length of MSNs and the spine density. The changes in the structure of striatal MSNs may contribute to the motor dysfunctions in PD (**Galli *et al*., 2014**).

In case of neuroinflammation and neurodegeneration, the microglia gets activated and causes the death of nigral dopamine neurons. During the process of inflammation, the level of COX-2 dramatically gets up-regulated that led to the concept that inhibition of COX-2 can minimize the process of inflammation (**Choi *et***

***al*., 2009)**. COX-2 is present in the postsynaptic neuronal cell bodies and with the help of their potential downstream effectors like prostaglandin and free radicals, it causes toxicity. COX-2 may cause the oxidative damage through the oxidizing reactive species produced during the peroxidation activity **(Kaufmann *et al*., 1996)**. The interaction between the microglial cells and apoptotic neurons has been reported to promote the expression of COX-2 and synthesis of prostaglandins. COX-2 amplifies the inflammatory responses along with toxic effects and thus helps in the progression of the neurodegeneration **(Bartels *et al*., 2010)**.

Several epidemiological studies have suggested the role of conventional NSAID, ibuprofen in the risk reduction of the PD progression. Ibuprofen is an inhibitor of COX-2 and is known to reduce the microglial activation and synaptic plasticity **(Tripathi *et al*., 2017; Rogers *et al*., 2017)**. We have conducted the present study to find out the effect of cypermethrin on the dendritic morphology of experimental animals in the presence of ibuprofen. We have found the beneficial effects of ibuprofen against cypermethrin induced changes in the dendritic length and number of dendritic spines.

4.2. Materials and methods

4.2.1. Chemicals

Cypermethrin, dimethyl sulfoxide (DMSO), gluteraldehydehyde and ibuprofen were purchased from Sigma Aldrich, St. Louis, MO, USA. Neg-50 from Richard Allen Scientific (Kalamazoo, MI, USA). Dibutyl phthalate xylenes (DPX), diethyl ether, formaldehyde potassium dichromate and silver nitrate were obtained from Sisco Research Laboratories Private Limited (Mumbai, India). Xylene was obtained from Thermo Fisher Scientific Pvt. Ltd (Rockford, IL, USA). All other chemicals used in the experiment were of analytical grade and procured locally.

4.2.2. Animal treatment, Brain isolation, and section cutting

The description about animal treatment, isolation of brain and section cutting has been given in detail in the above section 3.2.2. Without any alteration similar methodology has been used here also.

4.2.3. Dendritic arborization, ramification and spine density analysis

Using the standard protocol morphological alterations analysis in neurons, such as dendritic arborization, ramification and spine density in the striatum was performed **(Naskar *et al*., 2013)**. The sharp surgical blades were used to cut the small blocks of striatum incubated in the Golgi fixative (5% potassium dichromate, 5% chloral hydrate, 3.2% formaldehyde, 1.5% glutaraldehyde and 1% dimethyl sulfoxide) for overnight. The brain was changed with fresh fixative for further two days. After completion of incubation in the Golgi fixative, the brain was changed with silver nitrate solution (0.75%) for another two days. The sections were dehydrated by alcohol (70%) and glass slides were used for mounting. The inverted microscope (100xs, oil immersion) or confocal microscope under phase contrast (60 X phase contrast imaging with z-stacking) was used for the visualization of sections (60 µm). Image J software was used for the counting of dendritic spines and morphometric analysis.

4.2.4. Statistical analysis

Data were statistically analyzed with the help of one-way analysis of variance (ANOVA) followed by Newman-Keuls posthoc test. If the value is less than 0.05 then the data was considered statistically significant. Data was represented in the form of percent of saline control (saline).

4.3. Results

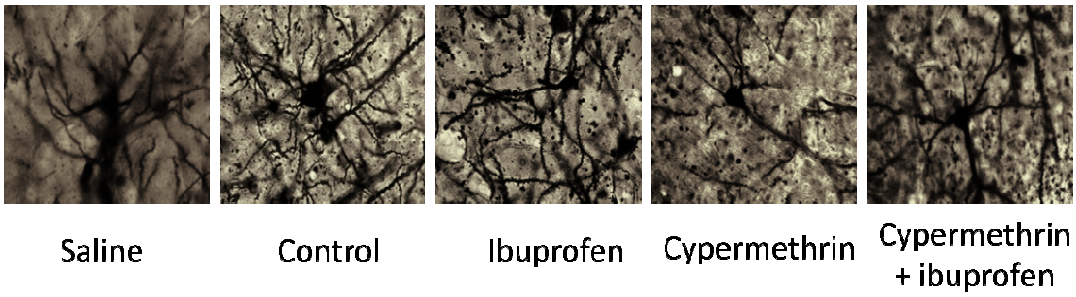
4.3.1. Striatal dendritic morphology and length

The dendritic length and the number of spines in the striatum of cypermethrin exposed animals were significantly reduced in comparison to control. Ibuprofen treatment significantly recovers the morphology and length of dendrites (Figure 4.1a, b). Ibuprofen alone did not affect the morphology and the length of dendrites.

4.3.2. Striatal dendritic spine number

The number of dendritic spines was found significantly decreased in cypermethrin treated animals. Dendritic spine numbers were found to be recovered towards control level upon ibuprofen treatment (Figure 4.2a, b).

(a)



(b)

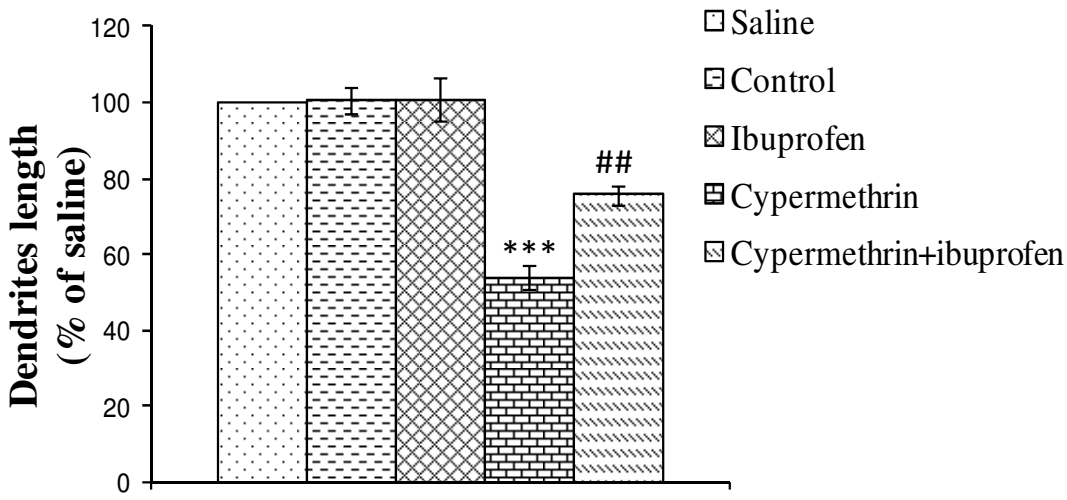
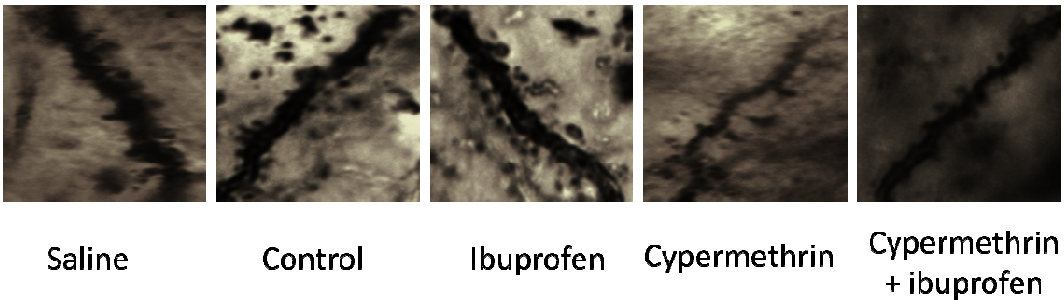


Figure 4.1: Effect of cypermethrin on dendritic morphology (a) and length of dendrites (b) in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated rats.

(a)



(b)

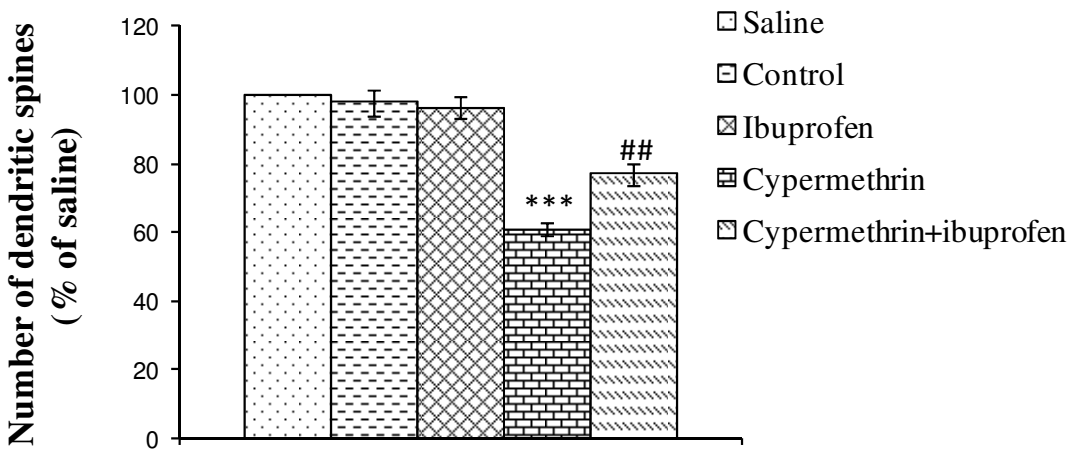


Figure 4.2: Effect of cypermethrin on morphology of dendritic spines (a) and number of dendritic spines (b) in the presence or absence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated rats.

Table 4.1: Effect of cypermethrin on the length of dendrites and number of spines in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) in comparison with cypermethrin-treated animals.

Group of animals	Length of dendrites	Number of spines
Saline	100.00±0.00	100.00±0.00
Control	100.68±3.33	97.87±3.98
Ibuprofen	100.78±5.81	96.44±3.00
Cypermethrin	53.92±3.26***	60.94±1.72***
Cypermethrin+ibuprofen	75.66±2.42##	76.84±3.33##

4.4. Discussion

In the present study, we have documented the morphological changes of dendrites of medium spiny neurons of striatum after cypermethrin treatment in rats in the form of reduced length and arborization and lessen a number of dendritic spines. Its because dendrites and dendritic spines are the places where majority of synaptic contact are present **(Solis *et al*., 2007)**. The simplest explanation of the present finding is that cypermethrin may have changed the synaptic inputs in the striatum resulting in the change in dendritic morphology. Our observations are in consistent with the previous findings on the changes in the dendritic morphology, length and spine numbers of dendrites of other toxin induced models **(Shin *et al*., 2016; Tripathi *et al*., 2017; Solis *et al*., 2007; Naskar *et al*., 2013)**. The exact mechanism by which cypermethrin induce changes in dendritic architecture is not clear. However, the depleted level of dopamine in PD may be responsible for this process. The level of connectivity and afferent activity depends on the dendritic arborization and spine

density present on dendrites. In the striatum, dopamine afferents make synapse at spine neck or dendritic shafts of medium spiny neurons **(Solis *et al*., 2007)**. Therefore we believe that the changes in dopaminergic inputs in the striatum may be responsible for the recognizable alterations in the morphology of dendrites and number of its spines. Previous studies have reported the COX-2 immunoreactivity in the dendritic spines. COX-2 contributes to the inflammation by playing role in the synthesis of prostaglandin. Chronic inflammation is very much associated with the pathogenesis of PD. Experimental studies have demonstrated that the activation of glial cells and release of proinflammatory cytokines is the common feature of PD brain. There is mounting evidence of rescuing effect of anti-inflammatory drugs on the development of PD **(Bartels *et al*., 2010)**.

In the present study, ibuprofen is examined for its therapeutic potential against pathogenesis of PD. This was an effort to find the additional beneficial effect of ibuprofen, if any, especially in the cypermethrin induced changes in the dendritic morphology and dendritic spine number. The major finding of the present work is the ability of ibuprofen is to attenuate and correct the dendritic morphology and dendritic spine loss of medium spiny neurons of striatum caused by cypermethrin. Ibuprofen is known to reduce inflammation by inhibiting the COX-2 activity because of its anti-inflammatory nature **(Hsieh *et al*., 2011)**. In cypermethrin induced model of PD ibuprofen minimizes the neuroinflammation and recovers the changes of dendritic morphology **(Singh *et al*., 2016)**. Ibuprofen recovers the length of dendrites of medium spiny neurons as well as the number of spines on dendrites in cypermethrin exposed rats.

Many reports are there that supports the fact that ibuprofen shows the protective effect against toxin induced models of PD **(Kurkowska-Jasterzebska *et al*., 2006;**

Hsieh *et al*., 2011). Depending on the previous reports it could be assumed that by normalizing the morphology of dendrites the complex behavioral abnormalities could be minimized.

Thus ibuprofen could be used as a rescuing agent to improve the dendritic morphology and spine density of medium spiny neurons by minimizing the inflammation. In short, ibuprofen could be used as a therapeutic agent for retarding the progression of PD.

CHAPTER 5

A crosstalk of ibuprofen-mediated alleviation in the cypermethrin-induced changes in nigral mitochondrial function and striatal dendritic arborization, ramification and spine density with anti-neuroinflammatory potential to delineate the mechanism of neuroprotection

CHAPTER 5

A crosstalk of ibuprofen-mediated alleviation in the cypermethrin-induced changes in nigral mitochondrial function and striatal dendritic arborization, ramification and spine density with anti-neuroinflammatory potential to delineate the mechanism of neuroprotection

5.1. Introduction

The characteristic anatomical feature of sporadic and pesticides-induced PD is the nigrostriatal dopaminergic neurodegeneration (**Barbeau *et al*., 1987; Koller *et al*., 1990**). The free radicals generated due to the cypermethrin intoxication leading to deterioration of nigral dopaminergic neurons thereby resulting in the PD phenotype in rodents (**Giray *et al*., 2001; Kale *et al*., 1999**). One of the most commonly used pesticides is pyrethroid that is metabolized in the brain by the xenobiotic metabolizing enzymes. Cypermethrin is known to induce oxidative stress, inflammation, and results in the slow progressive loss of nigrostriatal dopamine producing neurons. Cypermethrin is also responsible to induce microglial activation that could also contribute PD pathogenesis resulting to Parkinsonism (**Agrawal *et al.*, 2015b; Singh *et al*., 2016**).

In case of Parkinsonism increased level of cytokines and aberrant apoptosis occurs and it has been well established in toxin models of PD (**Srivastava *et al*., 2012; Dixit *et al*., 2013; Singh *et al*., 2016; Tripathi *et al*., 2017**). Depending on the intensity and activation event microglia can synthesize and release various kinds of free radicals, inflammatory cytokines and chemokines that are sufficient to induce an

inflammatory response. Morphologically activated microglia is the common feature of the chronic neurodegenerative disease and over the last years, it is the growing topic of research because the inflammation and microgliosis are known for their pathogenic roles **(Ajmone-Cat *et al.*, 2010)**.

Several experimental studies have explained the role of NSAIDs to provide protection against the loss of dopaminergic neurons in toxin induced Parkinsonian models **(Asanuma *et al*., 2006)**. Previous studies have mentioned that microglia is present in the substantia nigra in highest concentration. Thus the substantia nigra is more sensitive for the neuroinflammation. Microglial activation starts early in the neurodegeneration and starts at extending fibres like dendrites. Thus the level of detrimental compounds such as interleukin (IL-1β), interleukin (IL-4), interleukin (IL-17) and interferon-γ (IFN-γ) increases which may consequently induce inducible nitric oxide synthase (iNOS) or activates the process of apoptosis. It has been shown in many studies that overproduction of COX-2 during neuroinflammation causes the susceptibility to excitotoxicity while its inhibition offers neuroprotection **(Bartels *et al*., 2010)**. Consistently with the suggested role played by COX-2 in the neuronal excitotoxicity, COX-2 is also found to be expressed in the synaptic terminals of neurons.

NSAIDs agents possess the anti-inflammatory properties by inhibiting the COX-2 enzyme that is responsible for catalyzing the prostaglandin synthesis from the arachidonic acid **(Bartels *et al*., 2010)**. There are many NSAIDs are known but ibuprofen is preferred over others owing to the presence of prior epidemiological and experimental knowledge about its neuroprotective efficacy, cost effectiveness, nice public perception, general practice and relatively lesser toxicity after long-term exposure as compared with other NSAIDs of the similar efficacy **(Gao *et al*., 2008).**

However, the exact mechanism underlying the ibuprofen mediated protection in PD incidences is yet obscure.

In case of PD after the induction of inflammation, apoptosis also takes place in which many mitochondrial proteins are also associated. These mitochondrial proteins are known to be associated with the defective energy metabolism **(Pienaar *et al.*, 2010)**. Few studies have been done to find out the subtle mechanism involved in the process of cypermethrin induced dopaminergic neurodegeneration **(Chugh *et al.*, 1992; Nasuti *et al.*, 2007)**. Thus in order to find out the actual role of these proteins and how they are correlated with each other in PD, the present study was conducted. In PD progression to which extent inflammation is responsible and how the anti-inflammatory agents like ibuprofen could demise the process of neurodegeneration. Therefore it would be worthwhile to find out the role of ibuprofen against cypermethrin induced neurodegeneration.

5.2. Materials and methods

5.2.1. Chemicals

5-Bromo-4chloro-3-indolyl phosphate (BCIP), nitro-blue-tetrazolium (NBT), the primary antibody against β-actin and iNOS were purchased from Sigma Aldrich, St. Louis, MO, USA. Copper sulfate, diethyl ether, sodium dodecyl sulfate and sodium potassium tartrate were obtained from Merck India Private Limited, Mumbai, India. PVDF membrane was procured from Millipore (Billerica, MA, USA). Primary antibodies against anti-B-cell lymphoma (Bcl)-2, interleukin 1-β, interleukin-4 (IL-4), interleukin-17 (IL-17), interferon-gamma (IFN-γ), B-cell lymphoma extra large (Bcl-xL), Caspase-9, post- synaptic density protein 95 (PSD-95), synaptophysin (SYN), cytochrome c, mammalian translocase of inner mitochondrial membrane (mitochondrial import inner membrane translocase subunit; TIM44), anti-mouse, anti-rabbit and anti-goat alkaline phosphatase conjugated-secondary antibodies, were

purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-caspase-3 antibody was obtained from Cell Signalling Technology Inc. (Danvers, MA, USA). Glycerol, methanol, sodium carbonate and sodium thiosulfate were purchased from Merck Biosciences (Darmstadt, Germany). All other chemicals used in the study were of analytical grade and purchased locally.

5.2.2. Animal treatment, brain dissection and isolation of substantia nigra and striatum

The complete description of the animal treatment and dissection of brain has been given above in the section 3.2.2. The substantia nigra and striatum (substantia nigra and striatum= nigrostriatum) were isolated from the brain for performing the western blotting experiment.

5.2.3. Western blotting

The tissue isolated from substantia nigra and striatum was subjected to western blotting in order to find out the expression of some proteins related to neuroinflammation and apoptosis according to the protocol described elsewhere **(Tiwari et al., 2010)**. The supernatant was subjected to 8-15% SDS-PAGE after the homogenization, sonication and centrifugation of tissue. The expression pattern of caspase-3, caspase-9, Bcl-xl, cytochrome c (cytosolic and mitochondrial), β-actin, iNOS, synaptophysin and PSD-95 was analyzed from the supernatant. The nonspecific binding sites were blocked by 5% fat-free milk after protein transfer on PVDF membrane. The antibodies of anti-iNOS, anti-Bcl-2, anti-caspase-3, anti-caspase-9, anti-cytochrome-c and anti-β-actin (1:500 to 1:3,000 dilutions) were used for minimum 3 h to incubate the blots in it. The blots were washed after the completion of incubation in primary antibody and then incubated in anti mouse or anti rabbit-alkaline phosphatase conjugated secondary antibody. The blots were again washed with buffer and then developed in 5-Bromo-4-chloro--3′-indolyl

phosphate and nitro-blue tetrazolium solutions substrate. The image was captured with the help of computerized densitometry system (Alpha Imager System, Alpha Innotech Corporation, San Leandro, CA, USA). The loading control (β-actin) was used to normalize the band density of Bcl-2, Bcl-xl, caspase-3, caspase-9, cytochrome c (cytosolic), iNOS, interleukin-4 (IL-4), interleukin-17 (IL-17) and IL-1β. The TIM44 was used as loading control for mitochondrial cytochrome c to normalize band density **(Tiwari et al., 2010; Singh et al., 2016)**.

5.2.4. Protein estimation

Protein estimation of homogenate was done with the help of Lowry method and BSA was used as a standard **(Lowry *et al*., 1951).**

5.2.5. Statistical analysis

For the comparison purpose, one way analysis of variance (ANOVA) followed by Newman-Keuls posthoc test was used. Data were considered statistically significant if the value was less than 0.05. Representation of data was done as means ± Standard means error (SEM). Value of saline control was considered as 100% and other values were calculated accordingly.

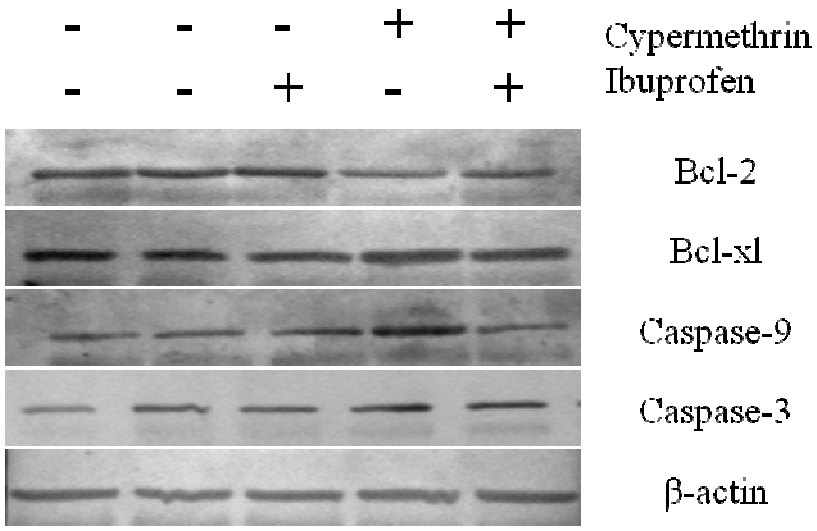
5.3. Results

5.3.1. Western blotting

Expression of proteins like Bcl-xL, caspase-3, caspase 9 (Figure 5.1a, b), cytochrome c (cytosolic) (Figure 5.2a, b), iNOS, IL-1β, IFN-γ, IL-4 and IL-17 (Figure 5.3a, b) in the nigrostriatal tissue of cypermethrin exposed rats were found to be increased in comparison with the control (Saline treated). The level of proteins like Bcl-2 (Figure 5.1a, b), cytochrome c (mitochondrial) (Figure 5.2a, b), striatal synaptophysin and PSD-95 (Figure 5.4a, b) were diminished after animals challenged with cypermethrin. Upon ibuprofen treatment the level of protein like Bcl-2, cytochrome c (mitochondrial) and synaptic proteins like synaptophysin and PSD-95 were found to

be up regulated while the expression of other nigrostriatal proteins like Bcl-xL, caspase-3, caspase-9, cytochrome c (cytosolic), iNOS, IL-1β, IFN-γ, IL-4 and IL-17 got down regulated toward the control level.

(a)



(b)

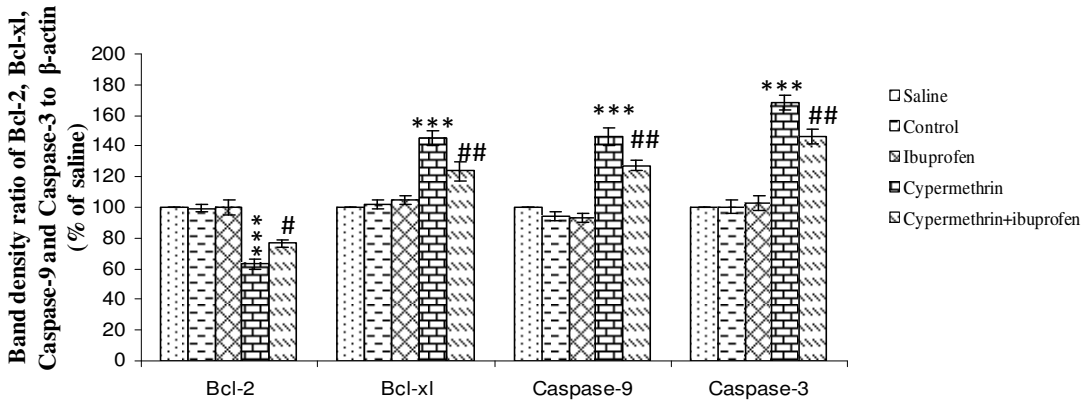
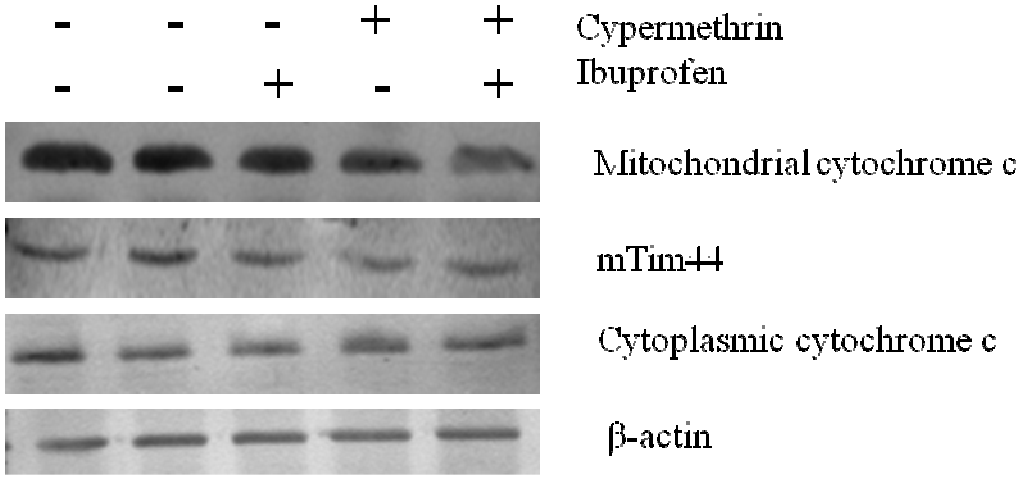


Figure 5.1: Effect of cypermethrin on the expression and band density ratio of Bcl-2, Bcl-xl, caspase-9 and caspase-3 with reference to β-actin proteins in the presence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$ in comparison with saline controls (saline) and ## ($p < 0.01$) and # ($p<0.05$) in comparison with cypermethrin-treated rats.

Table 5.1: Effect of cypermethrin on the Bcl-2, Bcl-xl, caspase-9 and caspase-3 in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$), # ($p<0.05$) in comparison with cypermethrin-treated animals.

Group of animals	Bcl-2	Bcl-xl	Caspase-9	Caspase-3
Saline	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Control	99.49±2.18	102.03±3.17	94.52±2.76	100.12±4.25
Ibuprofen	100.09±4.88	104.80±2.44	93.20±2.59	102.84±4.55
Cypermethrin	63.23±3.29***	145.28±5.06***	146.13±5.54***	168.35±4.80***
Cypermethrin+ibuprofen	76.52±2.33##	123.61±5.96##	127.10±3.35##	146.12±5.11##

(a)



(b)

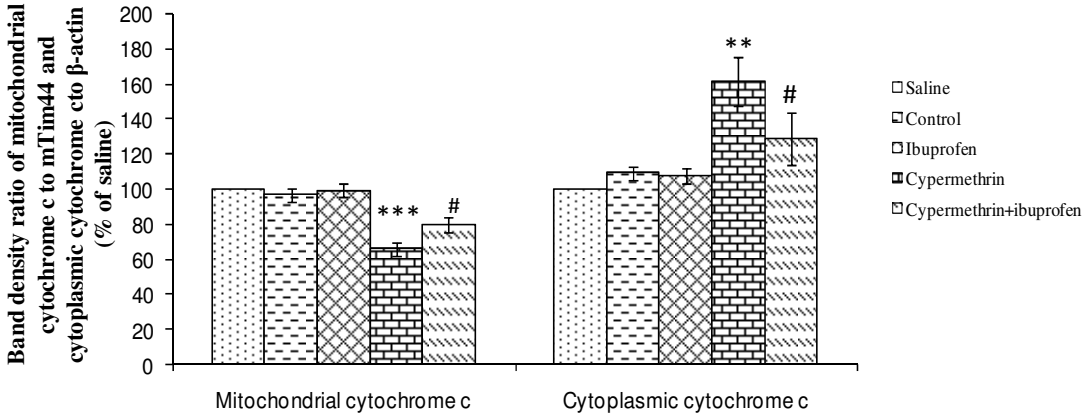
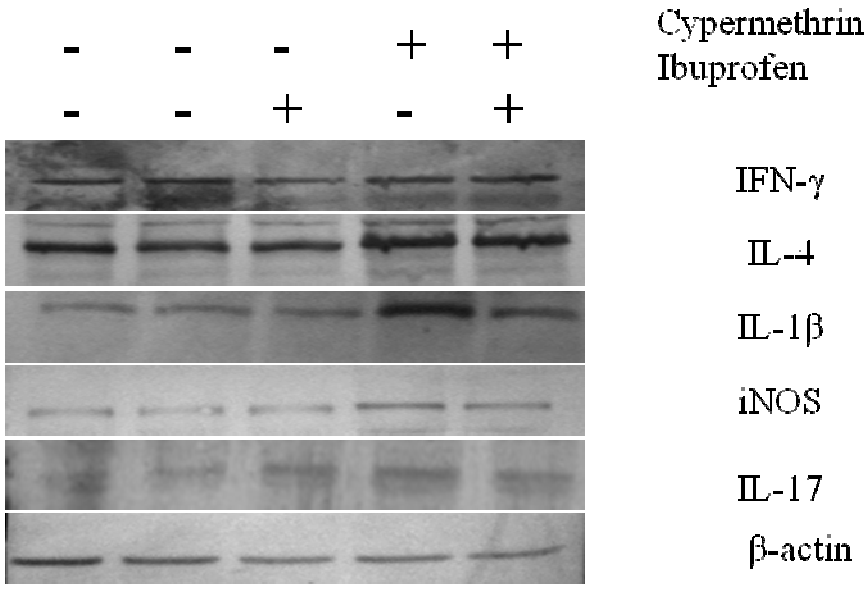


Figure 5.2: Effect of cypermethrin on the expression and band density ratio of mitochondrial cytochrome c with reference to mitochondrial Tim44 protein and expression and band density ratio of cytosolic cytochrome c with reference to β-actin protein in the presence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) and ** ($p < 0.01$) in comparison with saline controls (saline) and # ($p<0.05$) in comparison with cypermethrin-treated rats.

Table 5.2: Effect of cypermethrin on the mitochondrial cytochrome c and cytosolic cytochrome c in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) and ** ($p < 0.01$) in comparison with saline controls (saline) and # ($p<0.05$) in comparison with cypermethrin-treated animals.

Group of animals	Cytochrome c (Mitochondrial)	Cytochrome c (Cytosolic)
Saline	100.00±0.00	100.00±0.00
Control	96.10±3.97	108.98±3.92
Ibuprofen	98.63±3.85	107.24±4.32
Cypermethrin	65.43±4.13***	161.17±13.95**
Cypermethrin+ibuprofen	79.14±4.15#	128.25±14.95#

(a)



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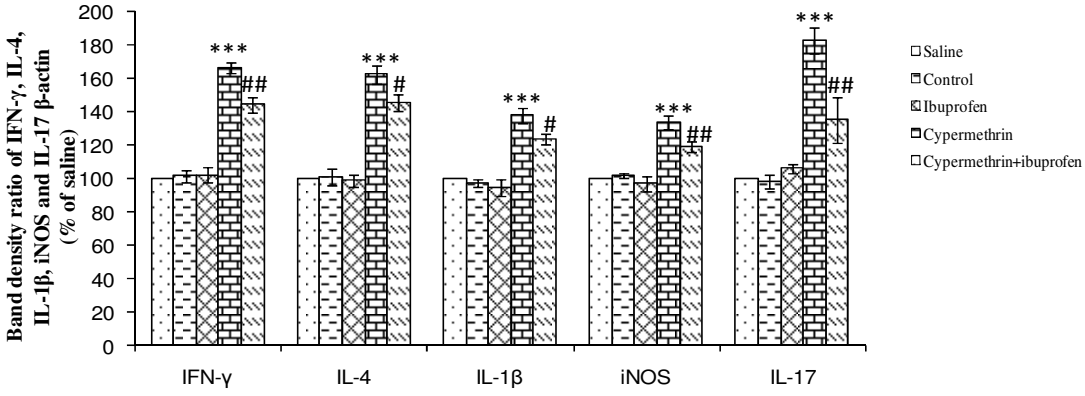
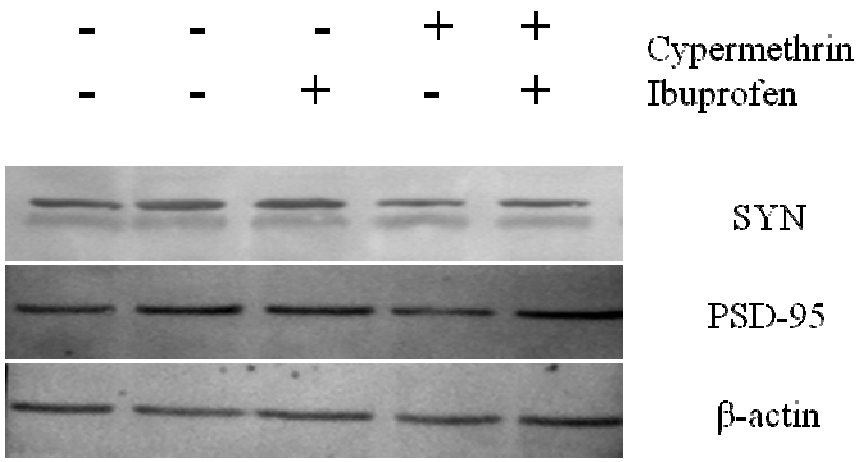


Figure 5.3: Effect of cypermethrin on the expression and band density ratio of IFN-γ, IL-4, IL-1β, iNOS and IL-17 with reference to β-actin protein in the presence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$), # ($p<0.05$) in comparison with cypermethrin-treated rats.

Table 5.3: Effect of cypermethrin on the IFN-γ, IL-4, IL-1β, iNOS and IL-17 in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$), # ($p<0.05$) in comparison with cypermethrin-treated animals.

Group of animals	IFN-γ	IL-4	IL-1β	iNOS	Il-17
Saline	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Control	101.01±3.52	100.43±4.93	96.92±1.99	101.22±1.51	97.87±3.98
Ibuprofen	101.60±4.44	98.19±3.28	93.99±5.12	96.37±4.19	105.50±2.88
Cypermethrin	165.94±2.86***	162.17±5.37***	137.47±4.40***	133.32±4.11***	182.46±7.89***
Cypermethrin+ibuprofen	143.83±4.37##	144.68±5.12#	123.38±3.10#	118.40±3.35##	134.80±13.71##

(a)



(B)

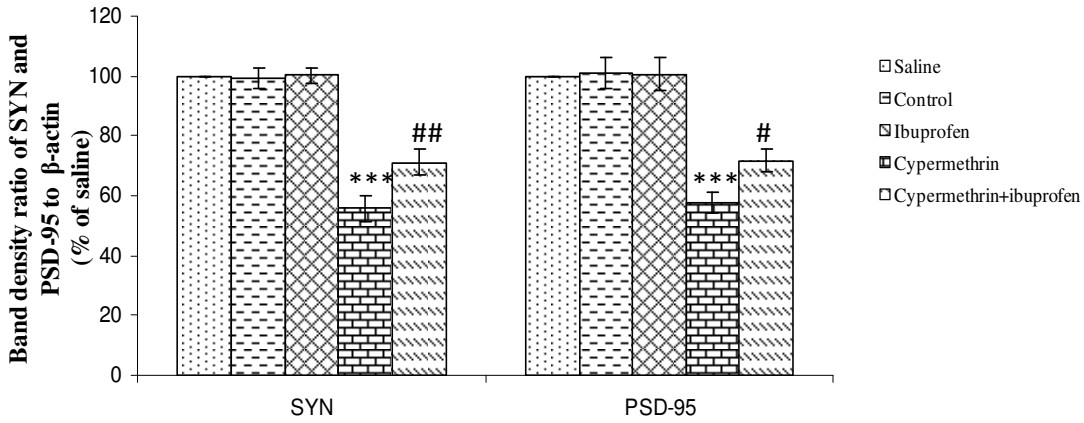


Figure 5.4: Effect of cypermethrin on the expression and band density ratio of SYN and PSD-95 with reference to β-actin protein in the presence of ibuprofen treatment. Values are calculated in means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) ## ($p < 0.01$), # ($p<0.05$) in comparison with cypermethrin-treated rats.

Table 5.4: Effect of cypermethrin on the synaptophysin and PSD-95 in the presence of ibuprofen treatment. The values are calculated in terms of % of saline control as means ± SEM (n = 3 independent experiments). Significant changes are expressed as *** ($p < 0.001$) in comparison with saline controls (saline) and ## ($p < 0.01$) and # ($p<0.05$) and in comparison with cypermethrin-treated animals.

Group of animals	Synaptophysin	PSD-95
Saline	100.00±0.00	100.00±0.00
Control	99.17±3.50	101.08±5.23
Ibuprofen	100.21±2.48	100.58±5.67
Cypermethrin	55.70±4.30***	57.84±3.56***
Cypermethrin+ibuprofen	71.18±4.14##	71.76±3.84#

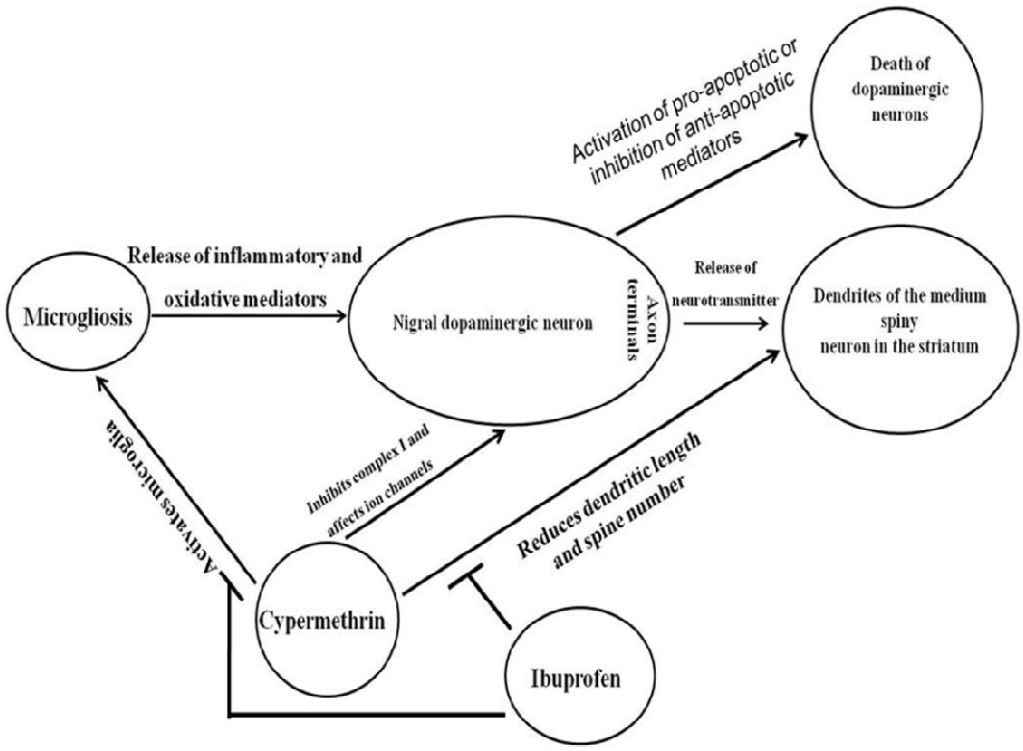


Figure 5.5: Flow chart representing the rescuing effect of ibuprofen against cypermethrin-induced Parkinsonism. Cypermethrin is known to induce the Parkinsonian features i.e. dopamine depletion, reduction in TH-immunoreactivity and increased microgliosis. Ibuprofen could provide protection against cypermethrin-induced changes. Moreover cypermethrin exposure causes decrease in dendritic length and number of spines and ibuprofen significantly rescues the changes significantly in the present study **(Tripathi *et al*., 2017)**.

5.4. Discussion

The hypothesis of neuroinflammation in the initiation and progression of the PD have been supported by many epidemiological studies (**Bartels *et al*., 2010; Wang *et al*., 2015**). Therefore it is desirable to develop some therapies that could intervene the inflammatory pathway mediated through microgliosis. Cypermethrin is known to responsible for the progressive loss of dopaminergic neurons (**Singh *et al*., 2012b; Agrawal *et al*., 2015b**). In case of PD terminal loss in the striatum has also been reported. COX-2 immunoreactivity is found in the dendritic spines present on dendrites and responsible for the signaling of the synapse (**Kaufmann *et al*., 1996**). Robust activation of microglial cells in the PD suggesting its importance for the progression of the pathogenesis of PD. Activated microglia is known to promote the COX-2 production and concomitant production of prostaglandin. COX-2 may produce toxic free radicals thereby promoting the progression of neurodegeneration. Microgliosis takes place in the substantia nigra and extends to dendrites and produces various detrimental compounds such as interleukins, interferon-γ (IFN-γ) and iNOS (**Bartels *et al*. 2010**).

As it is known that cypermethrin is responsible for the activation of the microglia and consequently COX-2 thus elevates the level of inflammation (**Singh *et al*., 2016**). In order to investigate the process of inflammation, we have checked the expression of some inflammatory compounds such as IL-1β, IL-4, IL-17, IFN-γ and iNOS. In cypermethrin exposed animals the expression of IL-1β, IL-4, IL-17, IFN-γ and iNOS was found to be increased (**Singh *et al*., 2016**). The process of inflammation leads to the degeneration of neurons. Thus in this sequence, we have also checked the expression of some proteins involved in the process of apoptosis. After cypermethrin treatment, the expression of Bcl-2, Bcl-xl, cytochrome-c

(cytosolic and mitochondrial), caspase-3 and caspase-9 was investigated. The expression of Bcl-2 and mitochondrial cytochrome c was found to be decreased while the expression of Bcl-xl, cytosolic cytochrome c, caspase-3 and caspase-9 was increased in cypermethrin exposed animals **(Singh *et al*., 2016; Agrawal *et al*., 2015a; Chauhan *et al*., 2015)**.

Synaptic terminals are known to play important role in the regulation and maintenance of neurotransmission of dopamine responsible for controlling the motor activity. Due to terminal degeneration in PD, it is important to check the expression of the synaptic proteins. SYN protein is a key signature protein of presynaptic terminal while PSD-95 is present at postsynaptic terminals and contributes to the regulation of synaptic strength **(Wheeler *et al*., 2002; Marqueze *et al*., 1991; Wiedenmann *et al*., 1985; Han *et al*., 2008; Bai *et al*., 2014; Shin *et al*., 2016)**. We have checked the expression of SYN and PSD-95 in the striatum. In cypermethrin intoxicated animals the level of SYN and PSD-95 was decreased. Thus it indicates that cypermethrin causes the loss of synaptic connectivity and intervene the dopaminergic neurotransmission in the striatum **(Tripathi *et al*. 2017)**.

Non-steroidal anti-inflammatory drugs have been known to provide the protective effect on dopaminergic neurons in PD. Ibuprofen, the conventional NSAID has the strongest epidemiological support for risk reduction of PD progression **(Bartels *et al*., 2010)**. Increased level of COX-2 in cypermethrin exposed animals was reduced by ibuprofen treatment and shows its anti-inflammatory nature **(Singh *et al*., 2016)**. Thus in the present study, we have used ibuprofen against cypermethrin induced toxicity and ibuprofen may provide protection due to its anti-inflammatory property. We have examined the expression of different inflammatory molecules such as IL-1β, IL-4, IL-17 and IFN-γ after ibuprofen treatment in cypermethrin treated animals.

Ibuprofen lowers the elevated level of inflammatory molecules (IL-1β, IL-4, IL-17, and IFN-γ). Ibuprofen was also found to be effective by diminishing the induced level of Bcl-xl, cytosolic cytochrome c, caspase-3 and caspase-9 while uplifts the decreased level of Bcl-2 and mitochondrial cytochrome c, proteins involved in apoptotic pathway in cypermethrin treated animals **(Tripathi *et al*., 2017; Singh *et al*., 2016)**.

COX-2 that are specifically present at nerve terminals may also responsible for the terminal loss thus we have checked the effect of ibuprofen on the two synaptic proteins, SYN and PSD-95 in cypermethrin exposed animals. Ibuprofen increased the diminished level of SYN and PSD-95 in cypermethrin treated animals. Thus ibuprofen provides protection against cypermethrin induced toxicity in the nerve terminals **(Tripathi *et al.*, 2017)**.

Thus the present study supports the fact that inflammation plays a key role in the progression of dopaminergic neurodegeneration and ibuprofen reduces the risk because of its anti-inflammatory nature against cypermethrin treated rats (Figure 5.5).

SUMMARY

SUMMARY

PD is a common age-related chronic neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra. The degeneration of dopaminergic neurons leads to the striatal dopamine depletion and appearance of disease symptoms. Motor symptoms develop as the disease progresses with the concomitant loss of dopaminergic neurons in the nigrostriatal pathway. Epidemiological and experimental studies have shown an association of pesticides exposure with PD pathogenesis. Long term exposure to cypermethrin in rats is known to induce dopaminergic neurodegeneration in human and animals while low doses and short term exposures of cypermethrin is less toxic, the mechanism through which cypermethrin induces neurodegeneration is yet not clearly known. However oxidative stress, mitochondrial dysfunction, α-synuclein aggregation and inflammation are found to participate in the nigrostriatal dopaminergic neurodegeneration. The role of inflammation in the PD pathogenesis is also supported by the fact that cypermethrin induces an accumulation of activated microglia in the substantia nigra. Nonsteroidal anti-inflammatory drugs (NSAIDs) that include ibuprofen found to protect demise of dopaminergic neurons in a few toxin models through their anti-inflammatory and anti-oxidant property or due to both. Cypermethrin induced inflammation to play a major role in disease progression. On the other hand, ibuprofen could protect the cypermethrin induced loss of dopaminergic neurons. Ibuprofen was performed in this study since it is a commonly recommended and very cost effective drug. It inhibits COX-2 activity, which is responsible for prostaglandin biosynthesis. The protective potential of

ibuprofen on cypermethrin induced inflammatory damage, terminal loss and other parameter were therefore investigated in the present study.

Male pups (Wistar rats) were treated with the cypermethrin (1.5 mg/kg; twice a week), intraperitoneally for 5-19 days. After the completion of postnatal treatment, the animals were left untreated for two months. Upon adulthood, the animals were again treated with cypermethrin (15 mg/kg; twice a week) for 12 weeks along with respective controls. In a few sets of experiments, the animals were co-treated intraperitoneally with ibuprofen (20 mg/kg; daily) for 12 weeks along with the respective vehicle. Upon completion of the treatment, the animals were sacrificed. The brain was separated from the skull by the cervical dislocation. The isolated brain was either immediately used to dissect the striatum and the substantia nigra or stored at -80^0 C till further use.

Dopamine content was measured in the striatum of all treated groups along with controls by liquid chromatography-mass spectrometry. Coronal sections of the substantia nigra were used to perform, the NeuN/TH and integrin-αM immunoreactivities and sections from striatum were used to perform TH-immunoreactivity. Golgi staining was implicated to perform the morphological study of dendrites and spines of medium spiny neurons in the striatum. Mitochondrial complex I activity was measured to check the mitochondrial function. The expression of some selected inflammatory, apoptotic and terminal proteins was checked to investigate the underlying mechanism of toxicity and protection.

Cypermethrin reduced the dopamine level, TH-immunoreactivity in the striatum and the number of nigral NeuN/TH positive cells. Integrin-αM immunoreactivity was elevated in the cypermethrin treated rats. Dendritic length and spine number in the

striatum was significantly reduced in cypermethrin treated animals. Mitochondrial complex I activity was also inhibited in cypermethrin treated group. Cypermethrin enhanced the expression of (inflammatory proteins) IL-1β, IL-4, IL-17 and IFN-γ, (apoptotic proteins) BCL-xL, cytosolic cytochrome c, caspase-3 and caspase-9. Cypermethrin reduced the expression of Bcl-2, mitochondrial cytochrome c and expression of (terminal proteins) SYN and PSD-95.

Ibuprofen co-treatment significantly normalized the changes induced by cypermethrin. Ibuprofen recovered the loss in the level of dopamine and TH-immunoreactivity in the striatum. Moreover, the number of NeuN/TH positive cells was restored towards normalcy after ibuprofen treatment. Ibuprofen also reduced the integrin-αM immunoreactivity towards the normal level. The mitochondrial complex I activity was also normalized in ibuprofen co-treated animals. Ibuprofen protected from the loss of dopamine terminals and enhanced the dendrite length and spine number in the striatum. Ibuprofen co-treatment significantly reduced the level of inflammatory proteins (IL-1β, IL-4, IL-17 and IFN-γ) in cypermethrin treated animals. Additionally, ibuprofen reduced the level of Bcl-xL, cytosolic cytochrome c, caspase-3 and caspase-9 (pro-apoptotic proteins) and enhanced the level of Bcl-2 and mitochondrial cytochrome c (anti-apoptotic proteins). Ibuprofen was found to normalize the expression of the terminal proteins, such as SYN and PSD-95 in cypermethrin-exposed animals.

The results of the study show that ibuprofen protects from cypermethrin-induced apoptosis, inflammation, neurodegeneration and incidences of PD. The study for the first time shows that ibuprofen can be tested in clinical investigation to assess its neuroprotective potential. If everything seems alright it can be used as an additional therapy to encounter PD owing to its cost effectiveness and neuroprotective efficacy.

REFERENCES

REFERENCES

1. Abeliovich, A., Gitler, A.D., 2016. Defects in trafficking bridge Parkinson's disease pathology and genetics. *Nature*. 539 (7628), 207-216.

2. Agrawal, S., Dixit, A., Singh, A., Tripathi, P., Singh, D., Patel, D.K., Singh, M.P., 2015a. Cyclosporine A and MnTMPyP Alleviate α-Synuclein Expression and Aggregation in Cypermethrin-Induced Parkinsonism. *Mol Neurobiol.*, 52, 1619-28.

3. Agrawal, S., Singh, A., Tripathi, P., Mishra, M., Singh, P.K., Singh, M.P., 2015b Cypermethrin-induced nigrostriatal dopaminergic neurodegeneration alters the mitochondrial function: a proteomics study. *Mol Neurobiol.* 51, 448-65.

4. Ajmone-Cat, M.A., Bernardo, A., Greco, A., Minghetti, L., 2010. Non-Steroidal Anti-Inflammatory Drugs and Brain Inflammation: Effects on Microglial Functions. *Pharmaceuticals.* 3, 1949-1964.

5. Amor, S., Puentes, F., Baker. D., van der Valk, P., 2010. Inflammation in neurodegenerative diseases. Immunology. 129(2), 154-69.

6. Anderson, D.C., Cranford, R.E., 1979. Corticosteroids in ischemic stroke. *Stroke.* 10 (1), 68-71.

7. Anwar, K., 2003. Cypermethrin, a pyrethroid insecticide induces teratological and biological changes in young chick embryos. *Pakistan J. Biol. Sci.* 6, 1698-1705.

8. Arimoto, T., Choi, D.Y., Lu, X., Liu, M., Nguyen, X.V., Zheng, N., Stewart, C.A., Kim, H.C., Bing, G., 2007. Interleukin-10 protects against inflammation mediated degeneration of dopaminergic neurons in substantia nigra. Neurobiol Aging. 28(6), 894-906.

9. Asanuma, M., Miyazaki, I., 2007. Common anti-inflammatory drugs are potentially therapeutic for Parkinson's disease? *Exp Neurol.* 206(2), 172-178.

10. Asanuma, M., Miyazaki, I., 2006. Nonsteroidal anti-inflammatory drugs in Parkinson's disease: possible involvement of quinone formation. *Expert Rev Neurother.* 6(9), 1313-1325.

11. Ascherio, A., Zhang, S.M., Hernán, M.A., Kawachi, I., Colditz, G.A., Speizer, F.E., Willett, W.C., 2001. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Ann. Neurol.* 50, 56-63.

12. Aschner, M., 2000. Manganese: brain transport and emerging research needs. *Environ. Health Perspect.* 108, 429-432.

13. Bai, X., Strong, R., 2014. Expression of synaptophysin protein in different dopaminergic cell lines. *J Biochem Pharmacol Res.* 2, 185-190.

14. Bains, J.S., Shaw, C.A., 1997. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res Brain Res Rev.* 25(3), 335-358.

15. Banerjee, R., Starkov, A.A., Beal, M.F., Thomas, B., 2009. Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochim Biophys Acta.* 1792, 651-663.

16. Barbeau, A., Roy, M., and Bernier, G., 1987. Ecogenetics of Parkinson's disease: prevalence and environmental aspects in rural areas. *Can. J. Neurol. Sci.*14, 36–41.

17. Barichella., M., Cereda, E., Pezzoli, G., 2009. "Major nutritional issues in the management of Parkinson's disease". *Mov. Disord.* 24(13), 1881-1892.

18. Barron, KD., 1995. The microglial cell. A historical review. *J. Neurol. Sci.* 134, 57-68.

19. Bartels, A.L., Leenders, K.L., 2010. Cyclooxygenase and neuroinflammation in Parkinson's disease neurodegeneration. Curr Neuropharmacol. 8, 62-68

20. Bartels, A.L., Willemsen, A.T., Doorduin, J., de Vries, E.F., Dierckx, R.A., Leenders, K.L., 2010. [11C]-PK11195 PET: quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? *Parkinsonism Relat Disord.* 16(1), 57-59.

21. Batiste-Alentorn, M., Xamena, N., Velazquez, A., Creus, A., Marcos, R., 1986. Mutagenicity of the pyrethroid insecticide cypermethrin in Drosophila. *Mutagenesis.* 1, 343-346.

22. Beal, M.F., 2002. Oxidatively modified proteins in aging and disease. *Free Radic. Biol. Med.* 32(9), 797-803.

23. Bekris, L.M., Mata, I.F., Zabetian, C.P., 2010. The genetics of Parkinson disease. *J. Geriatr. Psychiatry. Neurol.* 23, 228-242.

24. Bernstein, A.I., Garrison, S.P., Zambetti, G.P., O'Malley, K.L., 2011. 6-OHDA generated ROS induces DNA damage and p53- and PUMA-dependent cell death. *Mol. Neurodegener.* 6, 2.

25. Betarbet, R., Sherer, T.B., Greenamyre, J.T., 2002. Animal models of Parkinson's disease. *Bioessays.* 24, 308-318.

26. Bezard, E., Yue, Z., Kirik, D., Spillantini, M.G., 2013. Animal models of Parkinson's disease: limits and relevance to neuroprotection studies. Mov Disord. 28(1), 61-70.

27. Bezard, E., Przedborski, S., 2011. A tale on animal models of Parkinson's disease. *Mov. Disord.* 26, 993-1002.

28. Bhunya, S.P., Pati, P.C., 1988. Genotoxic effects of a synthetic pyrethroid insecticide, cypermethrin, in mice in vivo. *Toxicol. Lett.* 41, 223-230.

29. Blesa, J., Trigo□Damas, I., Quiroga□Varela, A. and Rey, N.L. Animal Models of Parkinson's Disease. http://dx.doi.org/10.5772/63328. Downloaded from: http://www.intechopen.com/books/challenges-in-parkinson-sdisease

30. Block, M.L., Hong, J.S., 2007. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem. Soc. Trans.* 35, 1127-1132.

31. Block, M.L., Zecca, L., Hong, J.S., 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57-69.

32. Bloomquist, J.R., Soderlund, D.M., 1985. Neurotoxic insecticides inhibit GABA-dependent chloride uptake by mouse brain vesicles. *Biochem. Biophys. Res. Commun.* 133, 37-43.

33. Burke, R.E., O'Malley., K., 2013. Axon degeneration in Parkinson's disease. *Exp Neurol.* 246, 72-83.

34. Burney, S., Caulfield, J.L., Niles, J.C., Wishnok, J.S., Tannenbaum, S.R., 1999. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat. Res.* 424 (1-2), 37-49.

35. Cabras, P., Caboni, P., Cabras, M., Angioni, A. and Russo, M., 2002. Rotenone residues on olives and in olive oil. *J. Agric. Food Chem.* 50, 2576-2580.

36. Cahill, E., Salery, M., Vanhoutte, P., Caboche, J., 2014.Convergence of dopamine and glutamate signaling onto striatal ERK activation in response to drugs of abuse.. Front Pharmacol. 4, 172.

37. Calabresi, P., Picconi, B., Parnetti, L., Di Filippo, M., 2006. A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholinesynaptic balance. *Lancet Neurol.* 5, 974-983.

38. Cao, T., Thomas, T.C., Ziebell, J.M., Pauly, J.R., Lifshitz, J., 2012. Morphological and genetic activation of microglia after diffuse traumatic brain injury in the rat. *Neuroscience.* 225, 65-75.

39. Carlsson, A., Lindqvist, M., Magnusson, T., 1957. 3, 4-Dihydroxyphenylalanine and 5- hydroxyl tryptophan as reserpine antagonists. *Nature.* 180 (4596), 1200.

40. Casper, D., Yaparpalvi, U., Rempel, N., Werner, P., 2000. Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro. *Neurosci Lett*. 289, 201–204.

41. Castaño, A., Herrera, A.J., Cano, J., Machado, A., 1998. Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J Neurochem.* 70, 1584–1592.

42. Caudle, W.M., Richardson, J.R., Wang, M.Z., Taylor, T.N., Guillot, T.S, McCormack, A.L., Colebrooke, R.E., Di Monte, D.A., Emson, P.C., Miller, G.W., 2007. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J. Neurosci. 27, 8138-8148.

43. Chen, H., Jacobs, E., Schwarzschild, M.A., McCullough, M..L., Calle, E.E., Thun, M.J., Ascherio, A., 2005. Nonsteroidal anti-inflammatory drug use and the risk for Parkinson's disease. *Ann. Neurol.* 58, 963-967.

44. Chen, H., Zhang, S.M., Hern´an, M.A., 2003. "Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease," *Archives of Neurology*. 60, 1059–1064.

45. Chesselet, M.F., Fleming, S., Mortazavi, F., Meurers, B., 2008. Strengths and limitations of genetic mouse models of Parkinson's disease. Parkinsonism. *Relat. Disord. Suppl*. 2, S84- S87.

46. Chesselet, M.F., Richter, F., 2011. Modelling of Parkinson's disease in mice. *Lancet Neurol.* 10, 1108-1118.

47. Choi, S.H., Aid, S., Bosetti, F., 2009. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol. Sci*., 30, 174-181.

48. Chugh, Y., Sankaranarayanan, A., Sharma, P.L., 1992. MK-801 antagonizes the lethal action of centrally and peripherally administered cypermethrin in mice and rats. *J. Pharm. Pharmacol.* 44, 521–523.

49. Cicchetti, F., Lapointe, N., Roberge-Tremblay, A., Saint-Pierre, M., Jimenez, L., Ficke, B.W., Gross, R.E., 2005. Systemic exposure to paraquat and maneb models early Parkinson's disease in young adult rats. *Neurobiol. Dis.* 20, 360-371.

50. Cicchetti, F., Drouin-Ouellet, J., Gross, R.E., 2009. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? *Trends Pharmacol. Sci.* 30, 475-483.

51. Cohen, G., Heikkila, R.E., 1974. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J. Biol. Chem.* 249, 2447-2452.

52. Collins, L.M., Toulouse, A. and Nolan, Y.M., 2012. Inflammation in Parkinson's Disease: Causes and Consequences, Mechanisms in Parkinson's Disease - Models and Treatments, Dr. Juliana Dushanova (Ed.), ISBN: 978-953-307-876-2, InTech, Available from: http://www.intechopen.com/books/mechanisms-inparkinson-s-disease-models-and-treatments/inflammation-in-parkinson-s-disease-causes-and-consequences.

53. Crawford, M.J., Croucher, A., Hutson, D.H., 1981. Metabolism of cis- and trans-cypermethrin in rats: Balance and tissue retention study. *J. Agr. Food Chem.* 29, 130-135.

54. Dahlström, A., Fuxe, K., 1964. Localization of monoamines in the lower brain stem. Experientia. 20, 398-399.

55. Dai, D.F., Chiao, Y.A., Marcinek, D.J., Szeto, H.H., Rabinovitch, P.S., 2014. Mitochondrial oxidative stress in aging and healthspan. Longev. *Healthspan*. 3, 6.

56. Dauer, W., Przedborski, S., 2003. Parkinson's disease: mechanisms and models. *Neuron.* 39, 889-909.

57. Dawson, T., Mandir, A., Lee, M., 2002. Animal models of PD: pieces of the same puzzle? *Neuron.* 35, 219-22.

58. Dawson, T.M., Ko, H.S., Dawson, V.L., 2010. Genetic animal models of Parkinson's disease. *Neuron*. 66, 646-661.

59. Dexter, D.T., Carayon, A., Javoy-Agid, F., Agid, Y., Wells, F.R., Daniel, S.E., Lees, A.J., Jenner, P., Marsden, C.D., 1991. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain.* 114, 1953-1975.

60. Dickson, D.V., 2007. "Neuropathology of movement disorders". In Tolosa E, Jankovic JJ. Parkinson's disease and movement disorders. *Hagerstown, MD: Lippincott Williams & Wilkins. pp.* 271-283.

61. Dixit, A., Srivastava, G., Verma, D., Mishra, M., Singh, P.K., Prakash, O., Singh, M.P., 2013. Minocycline, levodopa and MnTMPyP induced changes in the mitochondrial proteome profile of MPTP and maneb and paraquat mice models of Parkinson's disease. *Biochim Biophys Acta*. 1832(8), 1227-40.

62. Dodd, C.A., Klein, B.G., 2009. Pyrethroid and organophosphate insecticide exposure in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease: an immunohistochemical analysis of tyrosine hydroxylase and glial fibrillary acidic protein in dorsolateral striatum. *Toxicol Ind Health.* 25, 25-39.

63. Duan, C.L., Su, Y., Zhao, C.L., Lu, L.L., Xu, Q.Y., Yang, H., 2005. The assays of activities and function of TH, AADC, and GCH1 and their potential use in ex vivo gene therapy of PD. *Brain Res. Brain Res. Protoc.* 16(1-3), 37-43.

64. Duty, S., Jenner, P., 2011. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br J Pharmacol.* 164, 1357-1391.

65. Eells, J.T., Dubocovich, M.L., 1988. Pyrethroid insecticides evoke neurotransmitter release from rabbit striatal slices. *J. Pharmacol. Exp. Ther.* 246, 514–521.

66. Elmore, S., 2007.Apoptosis: a review of programmed cell death. *Toxicol Pathol.* 35, 495-516.

67. Elwan, M.A., Richardson, J.R., Guillot, T.S., Caudle, W.M., Miller, G.W., 2006. Pyrethroid pesticide-induced alterations in dopamine transporter function. *Toxicol Appl Pharmacol.*, 211, 188-197.

68. Esposito, E., Di Matteo, V., Benigno, A., Pierucci, M., Crescimanno, G., Di Giovanni, G., 2007. Non-steroidal anti-inflammatory drugs in Parkinson's disease. *Exp Neurol.* 205(2), 295-312.

69. Fahn, S., Sulzer, D., 2004. Neurodegeneration and neuroprotection in Parkinson disease. NeuroRx. 1, 139-154.

70. Fahn, S., Sulzer, D., 2004. Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx.* 1, 139-154.

71. Favaloro, B., Allocati, N., Graziano, V., Di Ilio, C., De Laurenzi, V., 2012. Role of apoptosis in disease. *Aging (Albany NY).* 4, 330-349.

72. Fitzgerald, M.J.T., 1996. Basal ganglia. In: Fitzgerald MJT, ed. Neuroanatomy Basic and Clinical, 3rd Edn. London: WB Saunders Company Ltd. 247-255.

73. Floodstrom, S., Warngard, L., Lijunquist, S., Ahlborg, U.G., 1988. Inhibition of metabolic cooperation in vitro and enhanced enzyme altered foci incidence in rat liver by the pyrethroid insecticide fenvalerate. *Arch. Toxicol.* 61, 218-233.

74. Forno, L.S., 1996. Neuropathology of Parkinson's disease. *J. Neuropathol. Exp. Neurol.* 55, 259-272.

75. Frank-Cannon, T.C., Alto, L.T., McAlpine, F.E., Tansey, M.G., 2009. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener. 16, 4, 47.

76. Galli, S., Lopes, D.M., Ammari, R., Kopra, J., Millar, S.E., Gibb, A., Salinas, P.C. 2014. Deficient Wnt signalling triggers striatal synaptic degeneration and impaired motor behaviour in adult mice. *Nat Commun*., 5, 4992.

77. Gao, H.M., Hong, J.S., 2011. Gene-environment interactions: key to unraveling the mystery of Parkinson's disease. *Prog. Neurobiol.* 94, 1-19.

78. Gao, H.M., Liu, B., Zhang, W., Hong, J.S., 2003. Critical role of microglial NADPH oxidase derived free radicals in the in vitro MPTP model of Parkinson's disease. *FASEB J.* 17, 1954-1956.

79. Gao, H.M., Zhang, F., Zhou, H., Kam, W., Wilson, B., Hong, J.S., 2011. Neuroinflammation and α-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. *Environ. Health Perspect.* 119, 807-814.

80. Gao, X., Chen, H., Schwarzschild, M.A., Ascherio, A., 2011a. "Use of ibuprofen and risk of Parkinson disease," *Neurology*. 76, 863–869.

81. Gerfen, C.R., Herkenham, M, Thibault, J., 1987. The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. J Neurosci. 7, 3915–3934.

82. Gerhard, A., Watts, J., Trender-Gerhard, I., Turkheimer, F., Banati, R.B., Bhatia, K., Brooks, D.J., 2004. In vivo imaging of microglial activation with [11C] (R)-PK11195 PET in corticobasal degeneration. *Mov. Disord.* 19(10), 1221-1226.

83. Giasson, B.I., Lee V.M., 2001. Parkin and the molecular pathways of Parkinson's disease. *Neuron.* 31, 885-888.

84. Gilbert, M.E., Mack, C.M., Crofton, K.M., 1989. Pyrethroids and enhanced inhibition in the hippocampus of the rats. *Brain Res.* 477, 314-321.

85. Giray, B., Gurbay, A., Hincal, F., 2001. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by Vitamin E or allopurinol. *Toxicol. Lett.* 118, 139-146.

86. Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C., Gage, F.H., 2010. Mechanisms underlying inflammation in neurodegeneration. Cell. 19, 140(6), 918-34.

87. Glinka, Y., Tipton, K.F., Youdim, M.B., 1996. Nature of inhibition of mitochondrial respiratory complex I by 6-Hydroxydopamine. *J. Neurochem.* 66, 2004-2010.

88. Glinka, Y.Y., Youdim, M.B., 1995. Inhibition of mitochondrial complexes I and IV by 6- hydroxydopamine. *Eur. J. Pharmacol.* 292, 329-332.

89. Goetz CG. Charcot on Parkinson's disease. *Mov. Disord.* 1986, 1: 27-32.

90. Goetz, C.G., 2011. The history of Parkinson's disease: early clinical descriptions and neurological therapies. Cold Spring Harb Perspect Med. 1(1), a008862.

91. Greggio, E., Cookson, M.R., 2009. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN Neuro. 1.

92. Gupta, S.P., Patel, S., Yadav, S., Singh, A.K., Singh, S., Singh, M.P., 2010. Involvement of nitric oxide in maneb- and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? *Neurochem Res.* 35(8), 1206-1213.

93. Han, K., Kim, E., 2008. Synaptic adhesion molecules and PSD-95. *Prog Neurobiol*. 84, 263-283.

94. He, Y., Appel, S., Le, W., 2001. Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamineinjection into mouse striatum. Brain Res. 909(1-2), 187-93.

95. Herrera, A.J., Castan, A., Venero, J.L., Cano, J., Machado, A., 2000. The Single Intranigral Injection of LPS as a New Model for Studying the Selective Effects of Inflammatory Reactions on Dopaminergic System. *Neurobiology of Disease*. 7, 429–447.

96. Hornykiewicz, O., 1998. Biochemical aspects of Parkinson's disease. Neurology. 51, S2–S9.

97. Horowitz, M.P., Greenamyre, J.T., 2010. Gene-environment interactions in Parkinson's disease: the importance of animal modeling. *Clin. Pharmacol. Ther.* 88, 467-474.

98. Hsieh., Y.C., Mounsey, R.B., Teismann, P., 2011. MPP (+)-induced toxicity in the presence of dopamine is mediated by COX-2 through oxidative stress. *Naunyn Schmiedebergs Arch Pharmacol.* 384(2), 157-67.

99. Hunter, R.L., Dragicevic, N., Seifert, K., Choi, D.Y., Liu, M., Kim, H.C., Cass,
W.A., Sullivan,P.G., Bing,G.,2007.Inflammation induces mitochondrial dysfu nction and dopaminergic neurodegeneration in the nigrostriatal system. *J Neurochem.* 100(5), 1375-86.

100. Ingham, C.A., Hood, S.H., Arbuthnott, G.W., 1989. Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res. 503, 334–338.

101. Irwin, R.P., Nutt, J.G., Woodward, W.R. and Gancher, S.T., 1992. Pharmacodynamics of the hypotensive effect of levodopa in parkinsonian patients. *Clin Neuropharmacol.* 15(5), 365-374.

102. Jankovic, J., 2008. "Parkinson's disease: clinical features and diagnosis". *J. Neurol. Neurosurg. Psychiatr.* 79 (4), 368-376.

103. Jin, Y., Zheng, S., Pu, Y., Shu, L., Sun, L., Liu, W., Fu, Z., 2011. Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (Danio rerio). *Chemosphere.* 82, 398-404.

104. Kale, M., Rathore, N., John, S., Bhatnagar, D., 1999. Lipid peroxidation and antioxidant enzymes in rat tissues in pyrethroid toxicity: possible involvement of reactive oxygen species. *J. Nutr. Environ. Med.* 9, 37-46.

105. Kanthasamy, A.G., Kitazawa, M., Kanthasamy, A., Anantharam, V., 2005. Dieldrin-induced neurotoxicity: relevance to Parkinson's disease pathogenesis. *Neurotoxicology.* 26, 701-719.

106. Kaufmann, S.H., 2008. Elie Metchnikoff's and Paul Ehrlich's impact on infection biology. Microbes Infect. 10(14-15), 1417-9.

107. Kaufmann, W.E., Worley, P.F., Pegg, J., Bremer, M., Isakson, P., 1996. COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci U S A.* 93, 2317-2321.

108. Kim-Han, J.S., Antenor-Dorsey, J.A., O'Malley, K.L., 2011. The Parkinsonian mimetic, MPP+, specifically impairs mitochondrial transport in dopamine axons. J Neurosci. 31, 7212–7221.

109. Kirby, M.L., Castagnoli, K., Bloomquist, J.R., 1999. In vivo effects of deltamethrin on dopamine neurochemistry and the role of augmented neurotransmitter release. *Pestic. Biochem. Physiol.* 65, 160–168.

110. Kitada, T., Asakawa, S., Hattori, N., Matsumine H., Yamamura Y., Minoshima S., 1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* 392, 605–608.

111. Klein, C., Westenberger, A., 2012. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med.* 2(1), a008888.

112. Kocaman, A.Y., Topaktas, M., 2009. The in vitro genotoxic effects of a commercial formulation of alpha-cypermethrin in human peripheral blood lymphocytes. *Environ. Mol. Mutagen.*, 50, 27-36.

113. Koller, W., Vetere-Overfield, B., Gray, C., Alexander, C., Chin, T., Dolezal, J., Hassanein, R., Tanner, C., 1990. Environmental risk factors in Parkinson's disease. *Neurology*. 40, 1218-1221.

114. Kraft, A.D., Harry, G.J., 2011. Features of microglia and neuroinflammation relevant to environmental exposure and neurotoxicity. Int J Environ Res Public Health. 8(7): 2980-3018.

115. Kurkowska-Jastrzebska, I., Członkowski, A., Członkowska., 2006. A Ibuprofen and themouse model of Parkinson's disease. *Ann Neurol*. 59, 988–989.

116. Lane, E., Dunnett, S., 2008. "Animal models of Parkinson's disease and L-dopa induced dyskinesia: how close are we to the clinic?" *Psychopharmacology.* 199, 303-312.

117. Lang, A.E., Lozano, A.M., 1998. Parkinson's disease: second of two parts. *N. Engl. J. Med.*, 339, 1130-43.

118. Langston, J.W., Ballard, P., Tetrud, J.W., Irwin, I., 1983. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*. 219, 979-980.

119. Langston, J.W., Forno, L.S., Rebert, C.S. and Irwin, I., 1984. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Res.* 292, 390-394.

120. Larranaga, M.M., Anadon, A., Martinez, M.A., Martinez, M., Castellano, V.J., Diaz , M.J., 2003. 5-HT loss in rat brain by type II pyrethroid insecticides. *Toxicol. Ind. Health.* 19, 147-155.

121. Le, W.D., Xu, P., Jankovic, J., Jiang, H., Appel, S.H., Smith, R.G., Vassilatis, D.K., 2003. Mutations in NR4A2 associated with familial Parkinson disease. *Nat. Genet.*, 33, 85-89.

122. Lees, A.J., 2007. "Unresolved issues relating to the shaking palsy on the celebration of James Parkinson's 250th birthday". *Mov. Disord. 22 (Suppl 17)*, S327-34.

123. Liang, C.L., Wang, T.T., Luby-Phelps, K., German, D.C., 2007. Mitochondria mass is low in mouse substantia nigra dopamine neurons: implications for Parkinson's disease. Exp Neurol. 203, 370–380.

124. Liang, L.P., Huang, J., Fulton, R., Day, B.J., Patel, M., 2007. An orally active catalytic metalloporphyrin protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in vivo. *J. Neurosci.* 27, 4326-4333.

125. Lijic, E., Guzman, J.N., Surmeier, D.J., 2011. The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. *Neurobiol. Dis.* 43(2), 364-371.

126. Liu, B., 2006. Modulation of microglial pro-inflammatory and neurotoxic activity for the treatment of Parkinson's disease. *Aaps. J.* 8, E606-621.

127. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 193(1), 265-75.

128. Lull, M.E., Block, M.L., 2010. Microglial activation and chronic neurodegeneration. Neurotherapeutics. 7(4), 354-65.

129. Lynd-Balta, E., Haber, S.N., 1994a. The organization of midbrain projections to the striatum in the primate: sensorimotor-related striatum versus ventral striatum. Neuroscience. 59, 625–640.

130. Lynd-Balta, E., Haber, S.N., 1994b. The organization of midbrain projections to the ventral striatum in the primate. Neuroscience. 59, 609–623.

131. Manna, S., Bhattacharyya, D., Mandal, T.K., Dey, S., 2005. Neuropharmacological effects of alphacypermethrin in rats. *Indian J. Pharmacol.* 37, 18-20.

132. Manning-Bog, A.B., Langston, J.W., 2007. Model fusion, the next phase in developing animal models for Parkinson's disease. *Neurotox. Res.* 11, 219-240.

133. Manthripragada, A.D., Schernhammer, E.S., Qiu, J., Friis, S., Wermuth, L., Olsen, J.H., 2011. Non-steroidal anti-inflammatory drug use and the risk of Parkinson's disease. *Neuroepidemiology.* 36, 155–61.

134. Marchi, S., Giorgi, C., Suski, J.M., Agnoletto, C., Bononi, A., Bonora, M., De Marchi, E., Missiroli, S., Patergnani, S., Poletti, F., Rimessi, A., Duszynski, J., Wieckowski, M.R., Pinton, P., 2012. Mitochondria-ros crosstalk in the control of cell death and aging. *J Signal Transduct.* 2012, 329635.

135. Marquèze-Pouey, B., Wisden, W., Malosio, M.L., Betz, H., 1991. Differential expression of synaptophysin and synaptoporin mRNAs in the postnatal rat central nervous system. *J Neurosci.* 1991, 3388-33897.

136. Matsuda, W., Furuta, T., Nakamura, K.C., Hioki, H., Fujiyama, F., Arai, R., Kaneko, T., 2009. Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. J Neurosci. 29, 444–453.

137. Matsuda, W., Furuta, T., Nakamura, K.C., Hioki, H., Fujiyama, F., Arai, R., Kaneko,
T.,2009.Single nigrostriatal dopaminergicneurons form widely spread and hig hly dense axonalarborizations in the neostriatum. *J Neurosci.* 29(2), 444-53.

138. Matteo, V.D., Pierucci, M., Di Giovanni, G., Di Santo, A., Poggi, A., Benigno, A., 2006. Aspirin protects striatal dopaminergic neurons from neurotoxin-induced degeneration: an in vivo microdialysis study. *Brain Res.* 1095, 167–77.

139. McGeer, P.L., McGeer, E.G., 2004. Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat. Disord.* 10 (Suppl 1), S3-7.

140. Mihm, M.J., Schanbacher, B.L., Wallace, B.L., Wallace, L.J., Uretsky, N.J., Bauer, J.A., 2001. Free 3- nitrotyrosine causes striatal neurodegeneration in vivo. *J. Neurosci.* 21(11), RC149.

141. Miller, G.W., 2007. Paraquat: the red herring of Parkinson's disease research. *Toxicol. Sci.* 100, 1-2.

142. Minghetti, L., Ajmone-Cat, M.A., De Berardinis, M.A., De Simone, R., 2005. Microglial activation in chronic neurodegenerative diseases: roles of apoptoti c neurons and chronic stimulation. *Brain Res Brain Res Rev.* 48(2), 251-6.

143. Mochizuki, H., Goto, K., Mori, H., Mizuno, Y.1996. Histochemical detection of apoptosis in Parkinson's disease. *J. Neurol. Sci.*, 137, 120-123.

144. Mohanakumar, K.P., Muralikrishnan, D., Thomas, B., 2000. Neuroprotection by sodium salicylate against 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-induced neurotoxicity. *Brain Res.* 864, 281–90.

145. Mohiuddin, I., Chai, H., Lin, P.H., Lumsden, A.B., Yao, Q., Chen, C., 2006. "Nitrotyrosine and chlorotyrosine: clinical significance and biological functions in the vascular system". *J. Surg.Res.* 133 (2), 143-9.

146. Montgomery, E.B. Jr., 1995. Heavy metals and the etiology of Parkinson's disease and other movement disorders. *Toxicology.* 97(1-3), 3-9.

147. Moore, A.H. Bigbee, M.J. Boynton, G.E., Wakeham, C.M., Rosenheim, H.M., Staral, C.J., Morrissey, J.L., Hund, A.K., 2010. Non-Steroidal Anti-Inflammatory Drugs in Alzheimer's disease and Parkinson's disease: Reconsidering the Role of *Neuroinflammation Pharmaceuticals*. 3(6), 1812-1841.

148. Mosley, R.L., Hutter-Saunders, J.A., Stone, D.K., Gendelman, H.E., 2012. Inflammation and adaptive immunity in Parkinson's disease. Cold Spring Harb Perspect Med. 2(1), a009381.

149. Mosley, R.L., Benner, E.J., Kadiu, I., Thomas, M., Boska, M.D., Hasan, K., Laurie, C., Gendelman, H.E., 2006. Neuroinflammation, Oxidative Stress and the Pathogenesis of Parkinson's disease. *Clin Neurosci Res.* 6(5), 261-281.

150. Murakoshi, H., Trimmer, J.S., 1999. Identification of the Kv2.1 K+ channel as a major component of the delayed rectifier K+ current in rat hippocampal neurons. *J Neurosci.* 19, 1728-1735.

151. Nagatsu, T., Sawada, M., 2007. Biochemistry of post-mortem brains in Parkinson's disease: historical overview and future prospects. J. Neural. *Transm. Suppl.*72, 113-120.

152. Narahashi, T., Frey, J.M., Ginsburg, K.S., Roy, M.L., 1992. Sodium and GABA-activated channels as the targets of pyrethroids and cyclodienes. *Toxicol. Lett.* 64, 429-436.

153. Narahashi, T., 1996. Neuronal ion channels as the target sites of insecticides. *Pharmacol. Toxicol.* 78, 1–14.

154. Narendra, D., Tanaka, A., Suen, D.F., Youle, R.J., 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol.* 183, 795-803.

155. Naskar, A., Manivasagam, T., Chakraborty, J., Singh, R., Thomas, B., Dhanasekaran, M., Mohanakumar, K.P., 2013. Melatonin synergizes with low doses of L-DOPA to improve dendritic spine density in the mouse striatum in experimental Parkinsonism. *J Pineal Res*. 55, 304-312.

156. Nasuti, C., Gabbianelli, R., Falcioni, M.L., Di Stefano, A., Sozio, P., Cantalamessa, F., 2007. Dopaminergic system modulation, behavioral changes, and oxidative stress after neonatal administration of pyrethroids. *Toxicology*. 229(3), 194-205.

157. Nicholson, G., Pereira, A. C. and Hall, G. M., 2002. Parkinson's disease and anaesthesia. *British Journal of Anaesthesia*. 89 (6), 904-16.

158. Nishi, A., Kuroiwa, M., Shuto, T., 2011. Mechanisms for the modulation of dopamine D1 receptor signaling in striatal neurons. *Front. Neuroanat.* 5, 1-10.

159. Olanow, C.W., Tatton, W.G., 1999. Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* 22, 123-144.

160. Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., Torizuka, T., 2005. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann. Neurol.* 57, 168-75.

161. Pacher, P., Beckman, J.S., Liaudet, L., 2007."Nitric oxide and peroxynitrite in health and disease." *Physiol. Rev.*, 87 (1), 315.

162. Paglini, G., Kunda, P., Quiroga, S., Kosik, K., Caceres, A., 1998. Suppression of radixin and moesin alters growth cone morphology, motility, and process formation in primary cultured neurons. J Cell Biol. 143, 443–455.

163. Paisan-Ruiz, C., Jain, S., Evans, E.W., Gilks, W.P., Simon, J., van der Brug, M., 2004. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* 44, 595–600.

164. Parkinson, J., 2002. An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin. Neurosci.* 14(2), 223-36.

165. Patel, S., Sinha, A., Singh, M.P., 2007. Identification of differentially expressed proteins in striatum of maneb-and paraquat-induced Parkinson's disease phenotype in mouse. *Neurotoxicol. Teratol.* 29, 578-585.

166. Peng, J., Stevenson, F.F., Oo, M.L., Andersen, J.K., 2008. Iron-enhanced paraquat-mediated dopaminergic cell death due to increased oxidative stress as a consequence of microglial activation. *Free Radic. Bio. Med.* 46, 312-320.

167. Pienaar, I.S., Dexter, D.T., Burkhard, P.R., 2010. Mitochondrial proteomics as a selective tool for unraveling Parkinson's disease pathogenesis. *Expert. Rev. Proteomics*. 7, 205-226.

168. Pinto, M., Nissanka, N., Peralta, S., Brambilla, R., Diaz, F., Moraes, C.T., 2016.

Pioglitazone ameliorates the phenotype ofa novel Parkinson's disease mouse model by reducingneuroinflammation. *Mol Neurodegener.* 2, 11, 25.

169. Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science.* 276, 2045–7.

170. Porras, G., Li, Q., Bezard, E., 2012. Modeling. Parkinson's Disease in Primates: The MPTP Model. *Cold Spring Harb Perspect. Med.* 2(3), a009308.

171. Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., Crews, F.T., 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia*. 55(5), 453-62.

172. Quik, M., O'Leary, K., Tanner, C.M., 2008. Nicotine and Parkinson's disease: implications for therapy. *Mov. Disord.* 23, 1641-1652.

173. Rao, G.V., Rao, K.S., 1997. Modulation of K+ transport across synaptosomes of rat brain by synthetic pyrethroids. *J Neurol Sci.* 147, 127-133.

174. Ray, D.E., Sutharsan, S., Forshaw, P.J., 1997. Actions of pyrethroid insecticides on voltage-gated chloride channels in neuroblastoma cells. *Neurotoxicology.* 18, 755-760.

175. Richardson, J.R., Caudle, W.M., Wang, M., Dean, E.D., Pennell., K.D., Miller, G.W., 2006. Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. *FASEB. J.* 20, 1695-7.

176. Ritz, B., Ascherio, A., Checkoway, H., Marder, K.S., Nelson, L.M., Rocca, W.A., Ross, G.W., Strickland, D., Van Den Eeden, S.K., Gorell, J., 2007. Pooled analysis of tobacco use and risk of Parkinson disease. *Arch. Neurol*. 64, 990-997.

177. Rocha, N.P., de Miranda, A.S., Teixeira, A.L., 2015. Insights into Neuroinflammation in Parkinson's Disease: From Biomarkers to Anti-Inflammatory Based Therapies. Biomed Res Int. 2015, 628192.

178. Rogers, J.T., Liu, C.C., Zhao, N., Wang, J., Putzke, T., Yang, L., Shinohara, M, Fryer, J.D., Kanekiyo, T., Bu, G., 2017. Subacute ibuprofen treatment rescues the synaptic and cognitive deficits in advanced-aged mice. *Neurobiol Aging.* 53, 112-121.

179. Sadek, H.L., Almohari, S.F., and Renno, W.M., 2014. The Inflammatory Cytokines in the Pathogenesis of Parkinson's Disease. *J Alzheimers Dis Parkinsonism*. 4, 3.

180. Sanchez-Pernaute, R., Ferree, A., Cooper, O., Yu, M., Brownell, A.L., Isacson, O., 2004. Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. *J Neuroinflammation*. 1, 6.

181. Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., 2009. Genomewide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet.* 41, 1303–7.

182. Schapira, A.H., Mann, V.M., Cooper, J.M., Dexter, D., Daniel, S.E., Jenner, P., Clark, J.B., Marsden, C.D., 1990. Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *J Neurochem.* 55, 2142-2145.

183. Scheffler, I.E., 2001. A century of mitochondrial research: achievements and perspectives. *Mitochondrion.* 1, 3-31.

184. Shastry, B.S., 2001. Parkinson disease: etiology, pathogenesis and future of gene therapy. *Neurosci. Res.* 41, 5-12.

185. Shin, M.S., Jeong, H.Y., An, D., Lee., H.Y., Sung, Y.H., 2016. Treadmill exercise facilitates synaptic plasticity on dopaminergic neurons and fibers in the mouse model with Parkinson's disease. *Neurosci Lett.* 621, 28-33.

186. Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Jenner, P., Marsden, C.D., 1994. Glutathione-related enzymes in brain in Parkinson's disease. *Ann. Neurol.* 36, 356-361.

187. Simon-Sanchez, J., Schulte, C., Bras, J.M., Sharma, M., Gibbs, J.R., Berg, D., 2009. Genome-wide association study reveals genetic risk underlying Parkinson' disease. *Nat Genet.* 41, 1308–1312.

188. Singh, A., Tripathi, P., Prakash, O., Singh, M.P., 2016. Ibuprofen abates cypermethrin-induced expression of pro-inflammatory mediators and mitogen-activated protein kinases and averts the nigrostriatal dopaminergic neurodegeneration. *Mol Neurobiol*. 53, 6849-6858.

189. Singh, A.K., Tiwari, M.N., Dixit, A., Upadhyay, G., Patel, D.K., Singh, D., Prakash, O., Singh, M.P., 2011a. Nigrostriatal proteomics of cypermethrin-induced dopaminergic neurodegeneration: microglial activation-dependent and -independent regulations. *Toxicol Sci.* 122, 526-38.

190. Singh, A.K., Tiwari, M.N., Prakash, O., Singh, M.P., 2012a. A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. *Curr Neuropharmacol.* 10, 64-71.

191. Singh, A.K., Tiwari, M.N., Upadhyay, G., Patel, D.K., Singh, D., Prakash, O., Singh, M.P., 2012b. Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: postnatal exposure enhances the susceptibility during adulthood. *Neurobiol Aging.* 33, 404-15.

192. Singh, B.K., Kumar, A., Ahmad, I., Kumar, V., Patel, D.K., Jain, S.K., Singh, C., 2011b. Oxidative stress in zinc-induced dopaminergic neurodegeneration: implications of superoxide dismutase and heme oxygenase-1. *Free Radic Res.* 45(10), 1207-22.

193. Singh, C., Ahmad, I., and Kumar, A., 2007. Pesticides and metals induced Parkinson's disease: involvement of free radicals and oxidative stress. *Cell Mol. Biol. (Noisy-le-grand).*, 53(5), 19- 28.

194. Singh, K., Singh, S., Singhal, N.K., Sharma, A., Parmar, D., Singh, M.P., 2010. Nicotine- and caffeinemediated changes in gene expression patterns of MPTP-lesioned mouse striatum: Implications in neuroprotection mechanism. *Chem. Biol. Interact.* 185, 81-9.

195. Singh, S., Singh, K., Patel, S., Singh, C., Nath, C., Singh, M.P., 2008. Nicotine and caffeine-mediated modulation in the expression of toxicant responsive genes and vesicular monoamine transporter-2 in 1-methyl 4-phenyl-1, 2, 3, 6- tetrahydropyridine-induced Parkinson's disease phenotype in mouse. *Brain Res.* 1207, 193-206.

196. Singhal, N.K., Srivastava, G., Patel, D.K., Jain, S.K., Singh, M.P., 2011. Melatonin or silymarin reduces maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. *J. Pineal Res.* 50, 97-109.

197. Soderstrom, K.E., O'Malley, J.A., Levine, N.D., Sortwell, C.E., Collier, T.J., Steece-Collier, K., 2010. Impact of dendritic spine preservation in medium spiny neurons on dopamine graft efficacy and the expression of dyskinesias in Parkinsonian rats. *Eur J Neurosci.* 3, 478-490.

198. Solis, O., Limón, D., Flores-Hernández, J., Flores, G., 2007. Alterations in dendritic morphology of the prefrontal cortical and striatum neurons in the unilateral 6-OHDA rat model of Parkinson's disease. Synapse. 61(6), 450-8.

199. Somayajulu-Niţu, M., Sandhu, J.K., Cohen, J., Sikorska, M., Sridhar, T.S., Matei, A., Borowy-Borowski, H. and Pandey, S., 2009. Paraquat induces oxidative stress, neuronal loss in substantia nigra region and parkinsonism in adult rats: neuroprotection and amelioration of symptoms by water-soluble formulation of coenzyme Q10. *BMC Neurosci.* 10, 88.

200. Srivastava, G., Dixit, A., Yadav, S., Patel, D.K., Prakash, O., Singh, M.P., 2012. Resveratrol potentiates cytochrome P450 2 d22-mediated

neuroprotection in maneb- and paraquat-induced parkinsonism in the mouse. *Free Radic Biol Med.* 52(8), 1294-306.

201. Staatz, C.G., Bloom, A.S., Lech, J.J., 1982. A pharmacological study of pyrethroid neurotoxicity in mice. *Pest. Biochem. Physiol.* 17, 287-292.

202. Steindler, D.A., 2007. Stem cells, regenerative medicine, and animal models of disease. *ILAR J.* 48(4), 323-38.

203. Stewart, I., Schluter, P.J., Shaw, G.R., 2006. Cyanobacterial lipopolysaccharides and human health a review. *Environ. Health.* 5, 7.

204. Subramaniam, S.R., Chesselet, M.F., 2013. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. *Prog Neurobiol*. 107, 17-32.

205. Sugama, S., Yang, L., Cho, B.P., DeGiorgio, L.A., Lorenzl, S., Albers, D.S., Beal , M.F., Volpe, B.T., Joh, T.H., 2003. Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. *Brain Res.* 964, 288-294.

206. Sulzer, D., Bogulavsky, J., Larsen, K.E., Behr, G., Karatekin, E., Kleinman, M.H., Turro, N., Krantz, D., Edwards., R.H., Greene, L.A., Zecca, L., 2000. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. Proc. Natl. Acad. Sci. U.S.A. 97, 11869-11874.

207. Sulzer D, Zecca L2000. Intraneuronal dopamine-quinone synthesis: a review. Neurotox. Res. 1, 181-195.

208. Szarka, A., Bánhegyi, G., Sümegi, B., 2014. Mitochondria, oxidative stress and aging. *Orv Hetil*. 155, 447-452.

209. Takakusaki, K., Okumura, T., 2008. Neurobiological Basis of controlling Posture and Locomotion. *Advanced Robotics.* 22, 1629-1663.

210. Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P. and Langston, J.W., 2011. Rotenone, paraquat, and Parkinson's disease. Environ. *Health Perspect.* 119, 866-872.

211. Tansey, M.G., Goldberg, M.S., 2010. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis. 37(3), 510-8.

212. Tareke, E., Bowyer, J.F., Doerge, D.R., 2007. Quantification of rat brain neurotransmitters and metabolites using liquid

chromatography/electrospraytandem mass spectrometry and comparison with liquid chromatography/electrochemical detection. *Rapid Commun Mass Spectrom.* 21(23), 3898-3904.

213. Tatton, N.A., 2000. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp. Neurol.* 166, 29-43.

214. Tavosanis, G., 2012. Dendritic Structural Plasticity. *Inc. Develop Neurobiol.* 72, 73–86.

215. Tayebati, S.K., Di Tullio, M.A., Ricci, A., Amenta, F., 2009. Influence of dermal exposure to the pyrethroid insecticide deltamethrin on rat brain microanatomy and cholinergic/dopaminergic neurochemistry. *Brain Res.* 1301, 180-188.

216. Terzioglu, M., Galter, D., 2008. "Parkinson's disease: genetic versus toxin-induced rodent models," *FEBS. J.* 275, 1384-1391.

217. Thiruchelvam, M., Brockel, B.J., Richfield, E.K., Baggs, R.B., CorySlechta, D.A., 2000. Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? *Brain Res.* 873, 225-234.

218. Tieu, K., 2011. A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb Perspect. Med*, 1, a009316.

219. Tiwari, M.N., Singh, A.K., Agrawal, S., Gupta, S.P., Jyoti, A., Shanker., R., Prakash O., Singh, M.P., 2012. Cypermethrin alters the expression profile of mRNAs in the adult rat striatum: a putative mechanism of postnatal pre-exposure followed by adulthood re-exposure-enhanced neurodegeneration. *Neurotox Res.*, 22(4): 321-34.

220. Tiwari, M.N., Singh, A.K., Ahmad, I., Upadhyay, G., Singh, D., Patel, D.K., Singh, C., Prakash, O., Singh, M.P., 2010. Effects of cypermethrin on monoamine transporters, xenobiotic metabolizing enzymes and lipid peroxidation in the rat nigrostriatal system. *Free Radic Res.* 44, 1416-24.

221. Tohidpour, A., Morgun, A.V., Boitsova, E.B., Malinovskaya, N.A., Martynova, G.P., Khilazheva, E.D., Kopylevich, N.V., Gertsog, G.E., Salmina, A.B., 2017. Neuroinflammation and Infection: Molecular Mechanisms Associated with Dysfunction of Neurovascular Unit. Front Cell Infect Microbiol. 20, 7, 276.

222. Tranzer, J.P., Thoenen, H., 1973. Selective destruction of adrenergic nerve terminals by chemical analogues of 6-hydroxydopamine. *Experientia.* 29(3), 314-5.

223. Tripathi, P., Singh, A., Bala, L., Patel, D.K., Singh, M.P., 2017. Ibuprofen Protects from Cypermethrin-Induced Changes in the Striatal Dendritic Length and Spine Density. *Mol Neurobiol.* DOI 10.1007/s12035-017-0491-9.

224. Tsuji, T., Asanuma, M., Miyazaki, I., Miyoshi, K., Ogawa, N., 2009. Reduction of nuclear peroxisome proliferator-activated receptor gamma expression in methamphetamine-induced neurotoxicity and neuroprotective effects of ibuprofen. *Neurochem Res*. 34, 764–774.

225. Ullah, M.S., Ahmad, M., Ahmad, N., Khan, M.Z., Ahmad, I., 2006. Toxic effects of cypermethrin in female rabbits. *Pakistan Vet. J.* 26, 193-196.

226. Ur Rasheed, M.S., Tripathi, M.K., Mishra, A.K., Shukla, S., Singh, M.P., 2016. Resveratrol Protects from Toxin-Induced Parkinsonism: Plethora of Proofs Hitherto Petty Translational Value. Mol Neurobiol. 53(5), 2751-2760.

227. Uversky, V.N., Li, J., Fink., A.L., 2001. Pesticides directly accelerate the rate of alpha-synuclein fibril formation: a possible factor in Parkinson's disease. *FEBS Lett.* 500, 105-108.

228. Uversky, V.N., 2004. Neurotoxicant-induced animal models of Parkinson's disease: understanding the role of rotenone, maneb and paraquat in neurodegeneration. *Cell Tissue Res.* 318, 225-241.

229. Van Den Eeden, S.K., Tanner, C.M., Bernstein, A.L., Fross, R.D., Leimpeter, A., Bloch, D.A., Nelson, L.M., 2003. Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. *Am. J. Epidemiol.* 157, 1015-1022.

230. Vila, M., Przedborski, S., 2003. Targeting programmed cell death in neurodegenerative diseases. *Nat. Rev. Neurosci.* 4, 365-375.

231. Villalba, R.M., Smith, Y., 2013.Differential striatal spine pathology in Parkinson's disease and cocaine addiction: a key role of dopamine? Neuroscience. 22, 251, 2-20.

232. Viswanath, V., Wu, Y., Boonplueang, R., Chen, S., Stevenson, F.F., Yantiri, F., Yang, L., Beal, M.F., Andersen, J.K., 2001. Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease. *J. Neurosci.* 21, 9519-9528.

233. Volpicelli-Daley, L.A., Luk, K.C., Patel, T.P., Tanik, S.A., Riddle, D.M., Stieber, A., Meaney, D.F., Trojanowski, J.Q., Lee, V.M., 2011. Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron. 72, 57–71.

234. Waldmeier, P., Bozyczko-Coyne, D., Williams, M., Vaught, J.L., 2006. "Recent clinical failures in Parkinson's disease with apoptosis inhibitors underline the need for a paradigm shift in drug discovery for neurodegenerative diseases," *Biochemical Pharmacology.* 72, 1197-1206.

235. Wang, Q., Liu, Y., Zhou, J. 2015. Neuroinflammation in Parkinson's disease and its potential as therapeutic target. *Translational Neurodegeneration*. 4, 19.

236. Wheeler, T.C., Chin, L.S., Li, Y., Roudabush, F.L., Li, L., 2002. Regulation of synaptophysin degradation by mammalian homologues of seven in absentia. *J Biol Chem*. 277, 10273-10282.

237. Wiedenmann, B., Franke., W.W., 1985. Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell*. 41, 1017-1028.

238. Wolansky, M.J., Harrill, J.A., 2008. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. *Neurotoxicol. Teratol.*, 30, 55-78.

239. Wooten, G.F., Currie, L.J., Bovbjerg, V.E., Lee, J.K., Patrie, J., 2004. Are men at greater risk for Parkinson's disease than women? *J. Neurol. Neurosurg. Psychiatry.* 75(4), 637-9.

240. Xiong, J., Zhang, X., Huang, J., Chen, C., Chen, Z., Liu, L., Zhang, G., Yang, J., Zhang, Z., Zhang, Z., Lin, Z., Xiong, N., Wang, T., 2016. Fenpropathrin a Widely Used Pesticide, Causes Dopaminergic Degeneration. Mol Neurobiol. *Mol Neurobiol.* 53(2), 995-1008.

241. Xiong, N., Huang, J., Zhang, Z., Zhang, Z., Xiong, J., Liu, X., Jia, M., Wang, F., Chen, C., Cao, X., Liang, Z., Sun, S., Lin, Z. and Wang, T., 2009. Stereotaxical infusion of rotenone: a reliable rodent model for Parkinson's disease. *PLoS One.* 4, e7878.

242. Yadav, S., Dixit, A., Agrawal, S., Singh, A., Srivastava, G., Singh, A.K., Srivastava, P.K., Prakash, O., Singh, M.P., 2012a. Rodent models and contemporary molecular techniques: notable feats yet incomplete explanations of Parkinson's disease pathogenesis. *Mol Neurobiol.* 46(2), 495-512.

243. Yadav, S., Gupta, S.P., Srivastava, G., Srivastava, P.K., Singh, M.P., 2012b. Role of secondary mediators in caffeine-mediated neuroprotection in maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. *Neurochem Res.* 37(4), 875-84.

244. Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* 44, 601–7.

245. AlDakheel, A., Kalia, L.V., Lang, A.E., 2014. Pathogenesis-targeted, disease-modifying therapies in Parkinson disease. Neurotherapeutics. 11(1), 6-23.

246. Lotharius, J., Brundin, P., 2002. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neurosci. 3, 932-942.

247. Barreto, G.E., Iarkov, A., Moran, V.E., 2015. Beneficial effects of nicotine, cotinine and its metabolites as potential agents for Parkinson's disease. Front Aging Neurosci. 9, 6, 340.

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ANNEXURE I

List of Publications

Annexure I

List of Publications

Publications

- **Tripathi P**, Singh A, Bala L, Patel DK, Singh MP (2017) Ibuprofen Protects from Cypermethrin-Induced Changes in the Striatal Dendritic Length and Spine Density. Mol Neurobiol. 55:2333-2339
- Singh A**,** **Tripathi P**, Prakash O, Singh MP (2016) Ibuprofen abates cypermethrin-induced expression of pro-inflammatory mediators and mitogen-activated protein kinases and averts the nigrostriatal dopaminergic neurodegeneration. Mol Neurobiol. 53:6849-6858
- Agrawal S, Dixit A, Singh A, **Tripathi P**, Singh D, Patel DK, Singh MP (2015). Cyclosporine A and MnTMPyP Alleviate α-Synuclein Expression and Aggregation in Cypermethrin-Induced Parkinsonism. Mol Neurobiol. 52:1619-28;
- Agrawal S, Singh A, **Tripathi P**, Mishra M, Singh PK, Singh MP (2015). Cypermethrin-induced nigrostriatal dopaminergic neurodegeneration alters the mitochondrial function: a proteomics study. Mol Neurobiol. 51:448-65
- **Tripathi P**, Singh A**,** Agrawal S, Prakash O, Singh MP (2014). Cypermethrin alters the status of oxidative stress in the peripheral blood: relevance to Parkinsonism. J Physiol Biochem. 70:915-924

Poster presented in international conference

- **Tripathi P**, Singh A, Bala L, Singh MP (2017). Does peripheral protein profile predict cypermethrin-induced nigrostriatal dopaminergic neurodegeneration? XVth Annual Conference of the Society for Free Radical Research (SFRR -2017) held at (BARC) Mumbai

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Ibuprofen Protects from Cypermethrin-Induced Changes in the Striatal Dendritic Length and Spine Density

Pratibha Tripathi[1] · **Ashish Singh**[1] · **Lakshmi Bala**[2] · **Devendra Kumar Patel**[3,4] · **Mahendra Pratap Singh**[1,3]

Received: 6 February 2017 / Accepted: 14 March 2017

Abstract Microgliosis and inflammation are major wrongdoers in cypermethrin-induced Parkinsonism along with oxidative stress, mitochondrial dysfunction and α-synuclein aggregation. Dopamine depletion could alter dendritic morphology, length and spine number in the striatum. Present study investigated the effect of ibuprofen on the dendritic morphology, length and spine density in cypermethrin PD model. Male pups were treated intraperitoneally with cypermethrin during postnatal days followed by adulthood to induce Parkinsonism using standard procedure along with controls. Subsets of animals were pre-treated with ibuprofen 2 h prior to cypermethrin treatment during adulthood. Standard methods were used to confirm Parkinsonism/neuroprotection. Striatal dendritic morphology, length, spine number and expression of synaptophysin and postsynaptic density protein-95 (PSD-95) along with the nigrostriatal pro-inflammatory and apoptotic proteins were measured. Cypermethrin induced Parkinsonian traits and attenuated the dendritic length, spine number and expression of synaptophysin and PSD-95. While cypermethrin increased the expression of interleukin-1β, interleukin-4, interferon-γ, inducible nitric oxide synthase, caspase-3, caspase-9 and B-cell lymphoma (Bcl)-xl proteins, it attenuated Bcl-2 expression. Ibuprofen normalized the changes in dendritic morphology, length, spine number and expression of synaptophysin, PSD-95, and pro-inflammatory and apoptotic proteins. Results demonstrate that cypermethrin induces inflammation and alters dendritic morphology, length and spine number, which are encountered by ibuprofen.

Keywords Parkinsonism · Dendritic morphology and spine number · Cypermethrin · Ibuprofen · Neuroprotection

Introduction

Parkinson's disease (PD) is a common and progressive dopamine depletion disorder of the central nervous system leading to motor disability [1]. Nigrostriatal dopaminergic neurodegeneration, microgliosis, mitochondrial dysfunction, inflammation, oxidative stress, aberrant autophagy, impaired ubiquitin proteasome system and deviant apoptosis have been linked with PD [2]. Substantia nigra is loaded with dopaminergic neurons while striatum is enriched with medium spiny neurons that receive dopaminergic signals through nerve terminals [3]. PD is labelled with selective loss of dopamine producing neurons in the substantia nigra and nerve terminals and synapses in the striatum. A scientific perception believes that the loss of synapse initiates neuronal demise and manifests the symptomatic depiction of PD.

Pesticides have been found to induce Parkinsonism in rodents after prolonged exposure [1, 4, 5]. Cypermethrin crosses the blood brain barrier and induces the nigrostriatal dopaminergic neurodegeneration and behavioural features of Parkinsonism [5]. Microgliosis indicates

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Ibuprofen abates cypermethrin-induced expression of pro-inflammatory mediators and mitogen-activated protein kinases and averts the nigrostriatal dopaminergic neurodegeneration

Ashish Singh[1] · **Pratibha Tripathi**[1] · **Om Prakash**[2] · **Mahendra Pratap Singh**[1]

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Abstract Cypermethrin induces oxidative stress, microglial activation, inflammation and apoptosis leading to Parkinsonism in rats. While ibuprofen, a non-steroidal anti-inflammatory drug, relieves from inflammation, its efficacy against cypermethrin-induced Parkinsonism has not yet been investigated. The study aimed to explore the protective role of ibuprofen in cypermethrin-induced Parkinsonism, an environmentally relevant model of Parkinson's disease (PD), along with its underlying mechanism. Animals were treated with/without cypermethrin in the presence/absence of ibuprofen. Behavioural, immunohistochemical and biochemical parameters of Parkinsonism and expression of pro-inflammatory and pro-apoptotic proteins along with mitogen-activated protein kinases (MAPKs) were determined. Ibuprofen resisted cypermethrin-induced behavioural impairments, striatal dopamine depletion, oxidative stress in the nigrostriatal tissues and loss of the nigral dopamine producing cells and increase in microglial activation along with atypical expression of pro-inflammatory and apoptotic proteins that include cyclooxygenase-2, tumour necrosis factor-α, MAPKs (c-Jun N-terminal kinase, p38 and extracellular signal-regulated kinase), B cell lymphoma 2-associated protein X, tumour suppressor protein p53, cytochrome c and caspase-3 in the nigrostriatal tissue. The results obtained thus demonstrate that ibuprofen lessens inflammation and regulates MAPKs expression thereby averts cypermethrin-induced Parkinsonism.

Keywords Parkinson's disease · Non-steroidal anti-inflammatory drug · Ibuprofen · Cypermethrin

Introduction

Parkinson's disease (PD) is a complicated and multi-factorial motor disorder of unexplained aetiology. It is mainly associated with movement mutilation and motor impairment owing to selective loss of the nigrostriatal dopamine synthesizing neurons [1–5]. Despite mysterious contributory factors, pesticides have been consistently accused in its pathogenesis [1–5]. Cypermethrin, a commonly used synthetic class II pyrethroid pesticide, is found to be safe at lower doses owing to its short half life. However, it may lead to progressive demise of dopaminergic neurons and onset of motor deficits in rats if it enters the brain and accumulates to a substantial level [6, 7]. Moderate doses of cypermethrin induce severity in adult rats, if pre-exposed to low doses during the postnatal periods [6, 7]. Directly or indirectly, the structural and functional impairments in the mitochondrion and microglial activation, inflammation, oxidative stress and defective apoptosis are found to be the foremost wrongdoers [6–10].

Mutation in inflammatory genes is found to be associated with PD pathogenesis. Besides, it triggers pro-inflammatory pathways resulting into the microglial activation and free radical generation [5, 11, 12]. Cypermethrin induces the microglial activation and overexpression of pro-inflammatory proteins, showing that inflammation could be critical in degeneration of the nigrostriatal dopaminergic neurons [8, 10]. However, it is not yet clear whether inflammation is a cause or consequence of cypermethrin-mediated

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Cyclosporine A and MnTMPyP Alleviate α-Synuclein Expression and Aggregation in Cypermethrin-Induced Parkinsonism

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Abstract Cypermethrin induces the mitochondrial dysfunction and oxidative damage to the nigrostriatal dopaminergic neurons leading to Parkinsonism in rats. Despite α-synuclein aggregation is reported to be critical in Parkinson's disease, its role and alliance with the mitochondrial dysfunction and oxidative damage leading to cypermethrin-induced Parkinsonism have not yet been deciphered. The present study aimed to examine the effect of cypermethrin on the expression and aggregation of α-synuclein and its subsequent connection with oxidative damage and mitochondrial dysfunction leading to the nigrostriatal dopaminergic neurodegeneration in the presence or absence of a mitochondrial membrane transition pore opening inhibitor, cyclosporine A and a superoxide dismutase/catalase mimetic, manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP). The expression of α-synuclein, 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE)-modified proteins, mitochondrial dysfunction-dependent apoptotic proteins, nitrite content, lipid peroxidation (LPO) and number of tyrosine hydroxylase (TH)-positive neurons were estimated in the substantia nigra and dopamine content in the striatum of control and treated rats employing standard procedures. Cypermethrin augmented the expression of α-synuclein, 3-NT, 4-HNE-modified proteins, caspase-3, mitochondrial Bax and cytosolic cytochrome-c along with nitrite and LPO and reduced the expression of cytosolic Bax, mitochondrial cytochrome-c, dopamine and number of TH-positive neurons. Cyclosporine A or MnTMPyP alleviated the expression and aggregation of α-synuclein along with indicators of the mitochondrial dysfunction, oxidative damage and dopaminergic neurodegeneration. The results demonstrate that cypermethrin induces α-synuclein expression and aggregation while cyclosporine A or MnTMPyP rescues from α-synuclein over-expression and aggregation along with the mitochondrial dysfunction and oxidative damage leading to Parkinsonism in rats.

Keywords α-Synuclein · Mitochondrial dysfunction · Oxidative damage, cypermethrin-induced Parkinsonism · Cyclosporine A · MnTMPyP

Introduction

Parkinson's disease (PD) is a chronic movement disorder of elusive aetiology and is characterized by the slow and progressive loss of dopaminergic neurons of the nigrostriatal pathway resulting in the depletion of the striatal dopamine and formation of Lewy bodies in the adjacent neurons [1, 2]. Lewy body is composed of a few abnormal and aggregated proteins that include α-synuclein, which is an incredibly small protein and is made up of 140 amino acids. Although α-synuclein is present in all neurons, it is highly abundant in presynaptic nerve terminals [1, 3]. Despite its tiny size, α-synuclein is reported to be critical in the onset and progression of familial PD. Recently, the decisive role of this ubiquitous protein in the pathogenesis of sporadic PD is also highlighted [1, 4]. Unlike mysterious aetiological factors of sporadic disease, few toxins are reported to induce secondary PD or Parkinsonism in experimental rodents. Neurotoxins, which include rotenone, paraquat, etc., also induce α-synuclein expression and aggregation in dopaminergic neurons of the nigrostriatal pathway similar to sporadic PD [1, 5].

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Cypermethrin-Induced Nigrostriatal Dopaminergic Neurodegeneration Alters the Mitochondrial Function: A Proteomics Study

Sonal Agrawal · Ashish Singh · Pratibha Tripathi · Manisha Mishra · Pradhyumna Kumar Singh · Mahendra Pratap Singh

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Abstract Cypermethrin induces the slow and progressive degeneration of the nigrostriatal dopaminergic neurons in rats. Postnatal preexposure with low doses of cypermethrin is known to enhance the susceptibility of animals upon adulthood reexposure. The study was undertaken to delineate the role of mitochondria in cypermethrin-induced neurodegeneration. Indexes of dopaminergic neurodegeneration, microglial activation, and mitochondrial dysfunction and its proteome profile were assessed in controls and cypermethrin-treated rats. Cypermethrin increased nigral dopaminergic neurodegeneration and microglial activation while reduced mitochondrial membrane potential and complex I activity. Cypermethrin attenuated striatal dopamine content and differentially regulated the expressions of the nine striatal and ten nigral proteins. Western blot analyses showed that cypermethrin also increased c-Jun N-terminal kinase (JNK), caspase-3, tumor suppressor protein (p53), tumor necrosis factor-α (TNF-α), p38 mitogen-activated protein kinase (p38 MAPK), and heme oxygenase-1 (HO-1) expressions and reduced B cell lymphoma-2 protein (Bcl-2) expression. Syndopa and minocycline rescued from cypermethrin induced augmentation in microglial activation and reductions in mitochondrial membrane potential and complex I activity, striatal dopamine content, and degeneration of nigral dopaminergic neurons. Syndopa and minocycline, respectively, modulated the expressions of four and six striatal and four and seven nigral proteins. Furthermore, they reinstated the expressions of JNK, caspase-3, Bcl-2, p53, p38 MAPK, TNF-α, and HO-1. The study demonstrates that cypermethrin induces mitochondrial dysfunction and alters mitochondrial proteome leading to oxidative stress and apoptosis, which regulate the nigrostriatal dopaminergic neurodegeneration.

Keywords Parkinson's disease · Mitochondrial dysfunction · Cypermethrin · Mitochondrial complex I · Mitochondrial proteome

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Introduction

Parkinson's disease (PD) is an aging-related chronic neurodegenerative disorder. It is characterized by the degeneration of the nigrostriatal dopaminergic neurons leading to the striatal dopamine deficiency [1]. The epidemiological and experimental studies have revealed a strong association between PD pathogenesis and exposures to pesticides and heavy metals [1, 2]. Cypermethrin, a synthetic class II pyrethroid insecticide, readily crosses the blood–brain barrier, enters the brain, and eventually leads to the nigrostriatal dopaminergic neurodegeneration in rats after prolonged exposure [3, 4]. Cypermethrin induces the progressive degeneration of dopaminergic neurons in adults; the degree of neuronal loss is found to be considerably increased when rats are preexposed with relatively low dose of cypermethrin during the critical period of the brain development [2].

Oxidative stress, mitochondrial dysfunction, altered energy metabolism, malfunctioning of the ubiquitin proteasome system, augmented apoptosis, impaired autophagy, and microglial activation are reported to be the critical players of PD pathogenesis [1]. The mitochondrial dysfunction theory of

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ORIGINAL PAPER

Cypermethrin alters the status of oxidative stress in the peripheral blood: relevance to Parkinsonism

Pratibha Tripathi · Ashish Singh · Sonal Agrawal · Om Prakash · Mahendra Pratap Singh

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Abstract Parkinson's disease (PD) is a motor scarcity disorder characterized by the striatal dopamine deficiency owing to the selective degeneration of the nigrostriatal dopaminergic neurons. While oxidative stress is implicated in PD, prolonged exposure to moderate dose of cypermethrin induces Parkinsonism. The study aimed to investigate the status of oxidative stress indicators and antioxidant defence system of the polymorphonuclear leukocytes (PMNs), platelets and plasma to delineate the effect of Parkinsonian dose of cypermethrin in the peripheral blood of rats and its subsequent relevance to Parkinsonism. Nitrite content, lipid peroxidation (LPO) and activity of superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione-*S*-transferase (GST) were measured in the PMNs, platelets and plasma of control and cypermethrin-treated rats in the presence or absence of a microglial activation inhibitor, minocycline or a dopamine precursor containing the peripheral 3,4-dihydroxyphenylalanine decarboxylase inhibitor, named syndopa, employing the standard procedures. The striatal dopamine was measured to assess the degree of neurodegeneration/neuroprotection. Cypermethrin increased nitrite and LPO in the plasma, platelets and PMNs while it reduced the striatal dopamine content. Catalase and GST activity were increased in the PMNs and platelets; however, it was reduced in the plasma. Conversely, SOD and GR activities were reduced in the PMNs and platelets but increased in the plasma. Minocycline or syndopa reduced the cypermethrin-mediated changes towards normalcy. The results demonstrate that cypermethrin alters the status of oxidative stress indicators and impairs antioxidant defence system of the peripheral blood, which could be effectively salvaged by minocycline or syndopa. The results could be of value for predicting the nigrostriatal toxicity relevant to Parkinsonism.

Keywords Parkinsonism · Peripheral blood · Cypermethrin · Minocycline · Syndopa

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Introduction

Parkinson's disease (PD) is a chronic and idiopathic neurodegenerative disorder characterized by the loss of dopaminergic neurons of the nigrostriatal pathway, which results in the postural instability, bradykinesia, resting tremor and muscular rigidity [8, 35]. Despite sporadic nature, ageing, genetic predisposition and environmental exposure to pesticides and metals are implicated in PD pathogenesis [8, 31, 35]. Epidemiological investigations revealed the role of dithiocarbamate, bipyridine and pyrethroid pesticides, which motivated researchers to measure the impact of these pesticides in rodents and also to develop animal models of PD. Several rodent models are developed, which possess a

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