FUNCTIONAL CHARACTERIZATION OF RISK FACTOR INVOLVED IN MPTP-INDUCED PARKINSONISM IN MICE

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All praises to **ALLAH Almighty**, the most beneficent who blessed mankind with a beautiful life and explorative nature. Humble dedications are for the most perfect personality of the universe of all the times **Prophet Hazrat Muhammad (PBUH).** The greatest scientist, whose moral and spiritual teachings enlightened our hearts, and flourished our thoughts towards achieving higher ideals of life.

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LIST OF ABBREVIATIONS

PD	**Parkinson's Disease**
MPTP	**1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine**
GFAP	**Glial fibrillary Acidic Protein**
SNpc	**Substantia Nigra Pars Compacta**
IHC	**Immunohistochemistry**
EOPD	**Early-Onset Parkinson's Disease**
RBD	**Rapid Eye Movement Sleep Behavior Disorder**
MAO-A	**Monoamine-A**
MAO-B	**Monoamine-B**
DAT	**Dopamine Transporter**
MPDP+	**1-methyl-4-phenyl-2,3-dihydropyridinium species**
I.V	**Intravenously**
S.C	**Subcutaneously**
TH	**Tyrosine Hydroxylase**

CNS	**Central Nervous System**
H &E	**Hematoxylin and Eosin**
CSF	**Cerebrospinal Fluid**

ABSTRACT

The second-most prevalent neurological disease in the world, Parkinson's disease (PD) affects roughly 4 million people. The death and loss of dopaminergic neurons in the substantia nigra compacta (SNpc) is the primary pathogenic characteristic of PD. Motor abnormalities include limb stiffness, tremor, and bradykinesia are the major features of PD. Although levodopa (L-DOPA) is the gold standard medication, but it has clear negative effects when taken over an extended period. Therefore, it is vital that novel medicines and ideal therapeutic agents be discovered. Parkinson's disease remains an unsolved clinical problem, as currently authorized PD therapies offer relatively modest therapeutic benefits. New therapeutic approaches that not only alleviate symptoms in the short term but also stop the disease from getting worse are desperately needed. For this purpose, mice model is utilized for PD induction by MPTP neurotoxin for functional characterization of proteomic factor GFAP as a risk factor in an effort to enhance the efficacy of the treatment of PD. In future, by targeting pathway of GFAP level in substantia nigra with some targeted drug will eventually lead to innovative therapeutic approach for PD patients.

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CHAPTER ONE: INTRODUCTION

1.1 Parkinson's Disease

Over 6 million individuals worldwide are affected by Parkinson's disease, the second most prevalent neurological condition. In the past 30 years, the occurrence of Parkinson's disease has risen by 2.5 times, making it one of the major neurological disabilities (Simon et al., 2020a). Parkinson's disease is characterized pathologically by Lewy and Lewy neurites, as well as neuronal death inside the substantia nigra as well as other parts of the brain. Lewy bodies are predominantly composed of accumulated and misfolded -synuclein species, which is why Parkinson's disease is classified as a synuclein pathology (Hayes, 2019).

Braak and colleagues proposed a model for the progression of Lewy disease, starting in the tail of brainstem and progressing rostrally through the higher brainstem, limbic areas, and eventually the neocortex. Such spread, however, presumably does not occur in all situations. Pathogens, cell-to-cell transmission, and the permissive templating of synuclein are examples of potential disease development routes (DISEASE Werner Poewe et al., n.d.).

Age is the key risk factor for Parkinson's disease, and males are more likely than women to develop the condition—the prevalence ratio is approximately three to one in favor of men. The development of Parkinson's disease in diverse populations has also been linked to a number of potentially changeable factors (such as toxins, water contaminants) and lifestyle factors (such as nicotine, caffeine, exercise, or head trauma). With more than 90 linked loci, illness risk also has a large genetic component (Schrag et al., 2019). Although tremendous strides have been made in our comprehension of the etiology and epidemiology, the origin of Parkinson's disease still seems to be unclear, and there is no recognized cure or preventative intervention at this time (Abbas et al., 2018).

1.2 Epidemiology

The incidence and prevalence of PD increase with age, affecting 1% of people over 65. The diagnosis of early-onset Parkinson's disease requires the beginning of Parkinsonian symptoms

before the age of 40. (EOPD). 3-5% of all PD cases are accounted for by it. It is separated into groups for "young-onset" and "juvenile" PD, which refer to cases that start before age 21 (Mehanna & Jankovic, 2019). Males have twice as much of a chance of having PD as females do in most populations. Apparently, female sex hormones have a protective effect. There may be unique genetic pathways to each gender or gender-specific differences in exposure that account for this gender difference in exposure to various risk factors (Dumurgier & Tzourio, 2020).

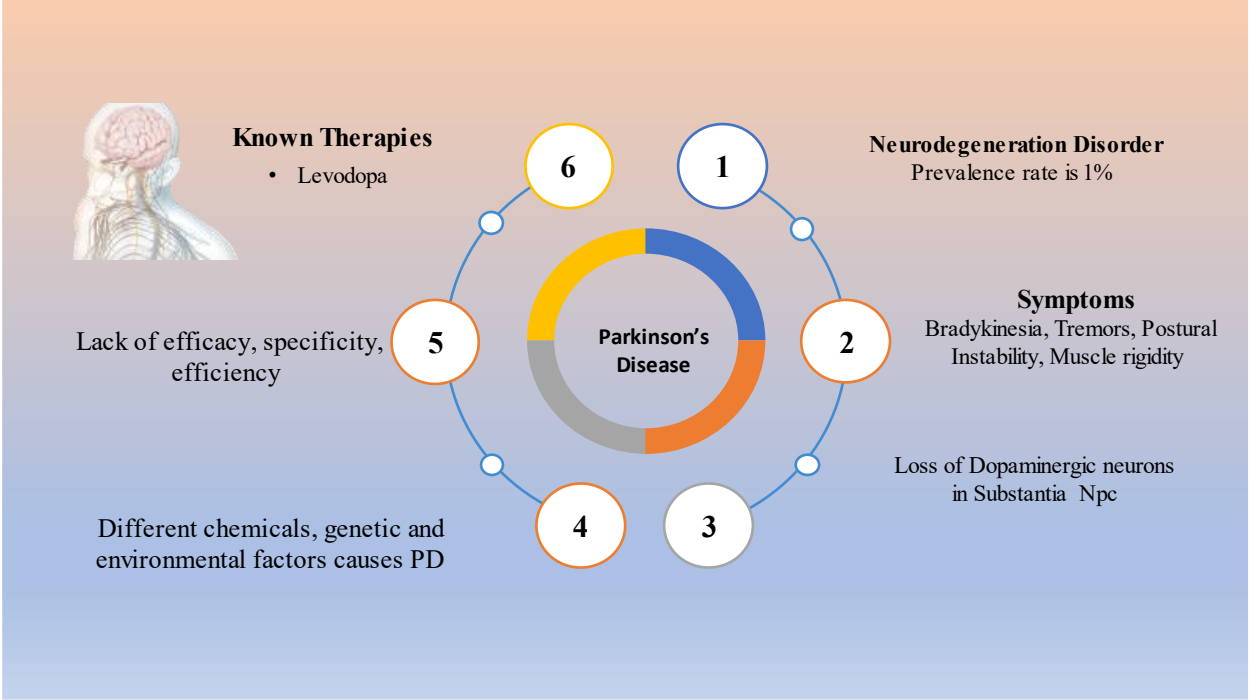

Figure: 1.1 Overview of Parkinson's Disease

1.2 Pathophysiology

The pathophysiology which includes the genetics as well as neurology of Parkinson's disease is as follows:

1.2.1 Genetics of PD

Only 5–10% of all instances of Parkinson's disease are genetic in nature. Existence of a family history, the disease's early start, and certain clinical characteristics (such as dystonia as a presenting

symptom) raise suspicions that a patient has the hereditary variant of the illness. More than 10% of YOPD patients have a genetic foundation, and if the disease manifests before the age of 30, the number of genetically characterized cases increases to more than 40% (Yu et al., 2018). Parkin (PARK2) as well as Leucine Rich Repeat Kinase 2 that includes (LRRK2/PARK8) and may include Alpha synuclein specifically (SNCA-PARK1/PARK4), as well as ATPase which includes Type 13A2 are some of the significant genes discovered as well as proven to be pivotal in Parkinson's (Simon et al., 2020b).

1.2.2 Neurology

In the substantia nigra pars compacta (SNpc) dopaminergic neurons are lost or degenerate in PD, and Lewy bodies, aberrant cellular clumps that contains proteins which includes alpha-synuclein as well as ubiquitin are accumulated. In the SNpc, between 60 and 70 percent of neurons are destroyed before to symptoms (Zesiewicz, 2019). The pathogenic process in PD not only affects the SNpc regions that contains dopaminergic neurons, but also affects the parts of the central as well as peripheral nervous systems, according to the research. As the disease progresses, limbic and neocortical brain regions are affected by pathology of Lewy bodies, which begins in neurons from different regions of the brainstem which contains the cholinergic as well as neurons from olfactory system (Bloem et al., 2020).

1.3 Clinical Diagnosis and Natural History

Over the past five years, clinical diagnostic criteria that are intended to improve Parkinson's disease diagnosis accuracy have been validated. A definitive diagnosis cannot be made from the earliest stages due to tests or biomarkers, and clinical features of this condition can overlap with those of other neurodegenerative diseases. As a result, even when the disease has clinically fully developed, clinical diagnostic accuracy is still below ideal (Tolosa et al., 2021).

Bradykinesia combined with at least one other symptom, such as muscle stiffness, rest tremor, or postural instability, that is the scientific definition of Parkinson's disease. The inception of motor symptoms is unilateral and includes the disease's asymmetry endures. The preponderance of

affected individual experiences as of non-motor symptoms. Some of these non-motor symptoms may appear years before the beginning of the main motor symptoms (Guerra et al., 2022).

These non-motor symptoms include pain also, mood disorders as well as cognitive impairment, and sleep disorders such as rambling as well as rapid eye movement sleep behavior disorder (RBD), and daytime drowsiness. They also include impaired sense of smell, disturbances in autonomous region such as postural hypotension, dysfunction of urogenital tract as well as constipation. According to the Sydney Multicenter research about Parkinson's disease, dementia (83%), hallucinosis (74%), symptomatic hypotension (48%), constipation (40%) and urine incontinence (20%) affected 71% of PD patients who had endured the condition for more than 20 years.[56] Additionally, 81%, 87%, and 48% of the patients exhibited freezing gait, postural instability, falling, and choking, respectively (Seppi et al., 2019).

Nearly ninety percent of PD patients develop non-motor symptoms during their illness, and these symptoms typically do not improve with dopamine medication. Constipation and depressive disorders practically quadruple a person's chance of getting PD in their later years. With idiopathic RBD, there is a significant chance that PD and other synuclein pithy may manifest. An average of 12 to 14 years pass in the middle of the development of RBD as well as the appearance of Parkinsonian motor symptoms (Rocha et al., 2022)

Age, disease severity, and greater dopaminergic drug dosages all correlate with an increase in the above-mentioned autonomic symptoms. Expulsion symptoms might include abundance as well as nocturia, urge incontinence, and urgency. Expulsion storage issues are more often than voiding issues. In comparison with Parkinson's disease (PD), expulsion symptoms are very common and manifest earlier in multisystem atrophy (MSA). Two-thirds of PD patients experience painful sensory symptoms, which are considered to result from faulty nociceptive processing (Rektorova, 2019).

Patients with Parkinson's disease are exposed to a six-fold greater risk of dementia, which arises later in the disease's course. Within 12 years of their diagnosis, up to 60% of PD patients get dementia. Ninety percent of people with inception of PD experience hyposmia, which often

appears years before typical motor symptoms. Hyposmia might indicate a greater chance of PD development, and olfactory testing could assist distinguish PD from other parkinsonian diseases (Zesiewicz, 2019).

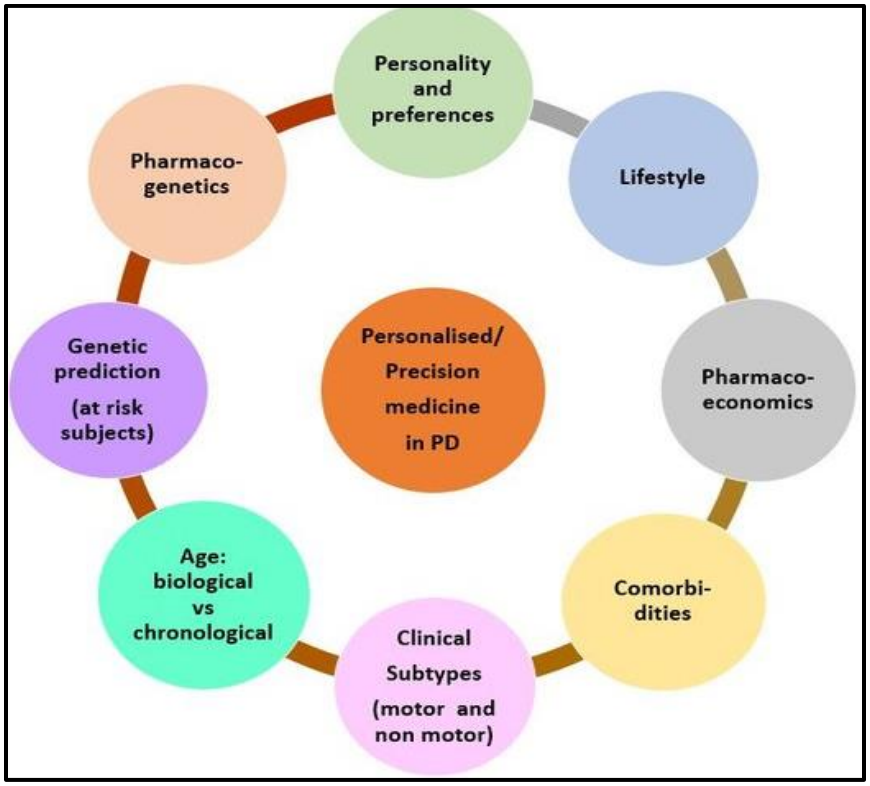

Figure: 1.2 Overview of PD that includes different ways such as personalized factors as well as some precision medicine to cure PD.

1.4 Pharmacologic Management

The development of disease-alternation therapies that can halt or may reduce the neuro-degenerative activity is the main goal of PD research. To accomplish this goal, there is currently no unambiguous disease-modifying medication.

1.4.1 Dopaminergic Therapy

Once patients experience functional impairment, the American Academy of Neurology (AAN) advises started keeping pharmacological regimens. Levodopa/carbidopa as well as dopamine agonists, or may include the monoamine oxidase-B (MAO-B) inhibitors as well as the injectable dopamine agonist (Zahoor et al., 2018b) catechol-O-methyltransferase (COMT) inhibitors, N-methyl-D-aspartate (NMDA) receptor inhibitors, and anticholinergics are among the medical treatments available for the treatment of Drug administration can be augmented by other pathways in the latter stages of PD which includes subcutaneous injections as well as intrajejunal infusions or may include the transdermal patches). The patient should consider deep brain stimulation if their dyskinesias and motor irregularities persist (DBS) (Rektorova, 2019).

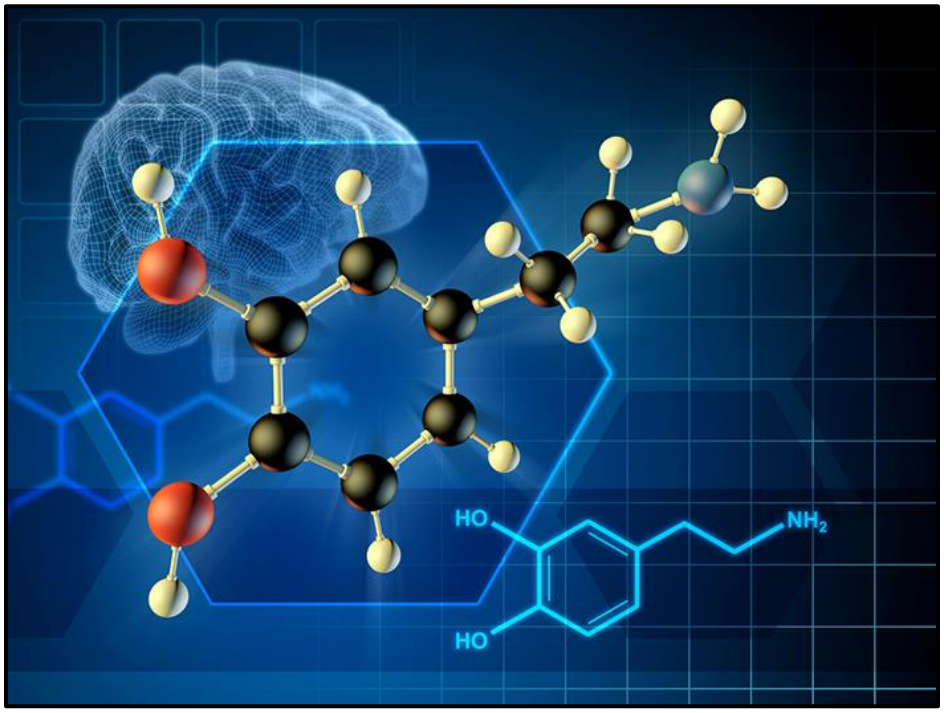

Figure: 1.3 Dopaminergic therapies that includes levodopa for the treatment of PD

In contrast to monoamine MAO B inhibitors, dopaminergic treatment is significantly more successful in treating bradykinesia and stiffness. Levodopa and dopamine agonists slow the course of illness and impairment. Trihexyphenidyl is an anticholinergic medication that can reduce

tremor, although dopamine replacement treatment has a weak and variable effect on tremor (Feng et al., 2020)

1.5 MPTP And Its Metabolites

The toxin MPTP shares structural similarities with several well-known environmental chemicals, such as the herbicide paraquat and the fish toxin/garden pesticide rotenone, both of which have been demonstrated to cause dopamine (DA) neuron degeneration.

When trying to create a synthetic version of heroin, a chemistry student in 1976 accidentally created MPTP, which kills dopaminergic (DAergic) neurons. In the early 1980s, others who were heroin addicts made the same error and experienced severe PD-like symptoms (Mustapha & Taib, 2021). The toxin potentiality of developing a reliable disease model was acknowledged by Dr. Langston, who cared for many of these patients. As soon as MPTP was administered to non-human primates, he and his colleagues were able to pinpoint its effects and explain the side effects that mimicked idiopathic PD's motor deficits. Sonsalla and Heikkila demonstrated in 1986 that mice might experience many of the same effects from MPTP (Bhurtel et al., 2019).

It is a universal consesus that astrocytes convert MPTP to its poisonous metabolite, the 1-methyl-4-phenylpyridinium (MPP+) ion. MPTP is highly lipophilic and readily crosses the blood brain barrier, where it binds mostly in astrocyte lysosomes (Ferrucci & Fornai, 2021a).

The blood brain barrier is not easily crossed by MPP+ because of its charge, hence systemic treatment does not harm central DAergic neurons. The DAergic nigrostriatal system is mostly destroyed by its direct infusion into the brain, albeit. The dopamine transporter (DAT) loves MPP+, which explains MPP+'s preference for DAergic neurons. MPP+ is a potent inhibitor of complex I respiration in isolated mitochondria, even though the mechanisms by which MPP+ induces cell death are not entirely understood (Ferrucci & Fornai, 2021b). As a result, the striatum and substantia nigra pars compacta (SNpc), the areas of the brain most vulnerable to MPTP-induced neurotoxicity, rapidly lose their adenosine triphosphate (ATP) concentration. It's interesting to note that twenty five percent inhibition of complex I can source a significant ATP

depletion. Contingenting on the regimen, DA neurons can be dead through both apoptosis as well as the necrosis.

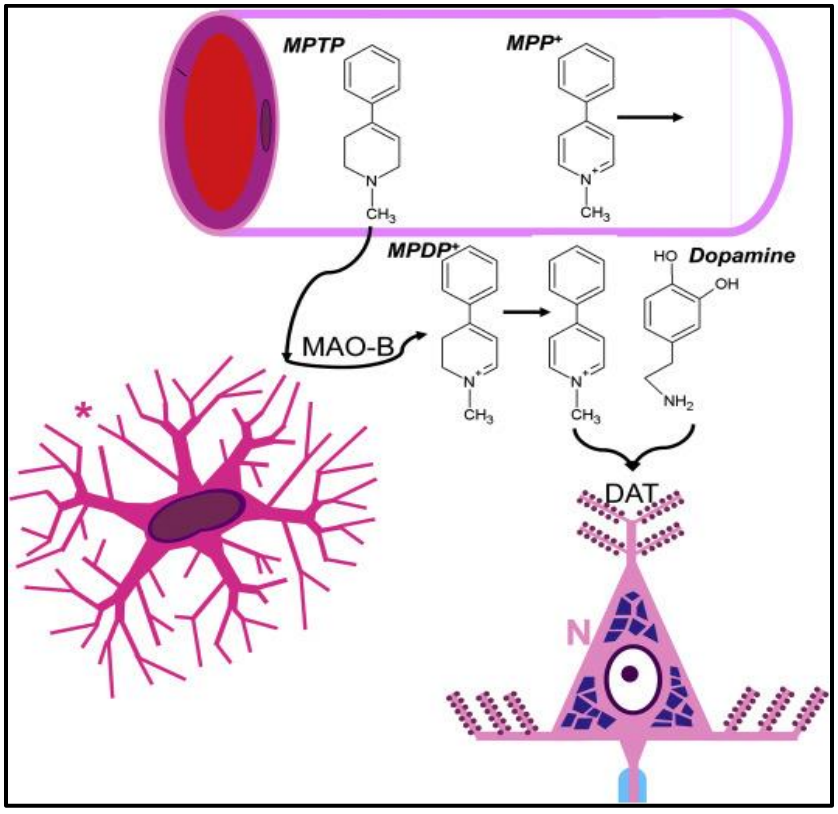

Figure: 1.4 Diagram depicting the stages of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic toxicity. Dopaminergic neurons (N) that express the dopamine transporter specifically take up MPP+, which is created when MPTP is oxidized by MAO-B in astrocytes (*). (DAT).

The hydro pyridine or may be its metabolite can be eliminated from the brain within twelve hours of exposure to MPTP or MPP+, as well as the ATP depletion cannot be prolonged detectable 24 hours after administration. The real neuronal loss, however, appears to take more time (Bourque et al., 2016). These findings mark up the idea that MPTP causes additional circumstances that eventually lead to the neurotoxicity.

1.6 Glial fibrillary acidic protein

GFAP, also known as glial fibrillary acidic protein, is an intermediate filament protein that emerged at initial stage in the progression of vertebrates, concurrently along with the emergence of several glial cell types in the central nervous systems (Ayala et al., 2021). Intermediate filaments are essential parts of the cytoskeleton in the cytoplasmic region and provide a variety of functions, including skeletal frame, scaffolding for organelles as well as that of enzymes and mechano-sensing of the outside of the cell's environment. GFAP is categorized as a type III intermediate filament along with vimentin as well as the desmin and also the peripherin that are actually neurons based on sequence homology (Middeldorp & Hol, 2011).

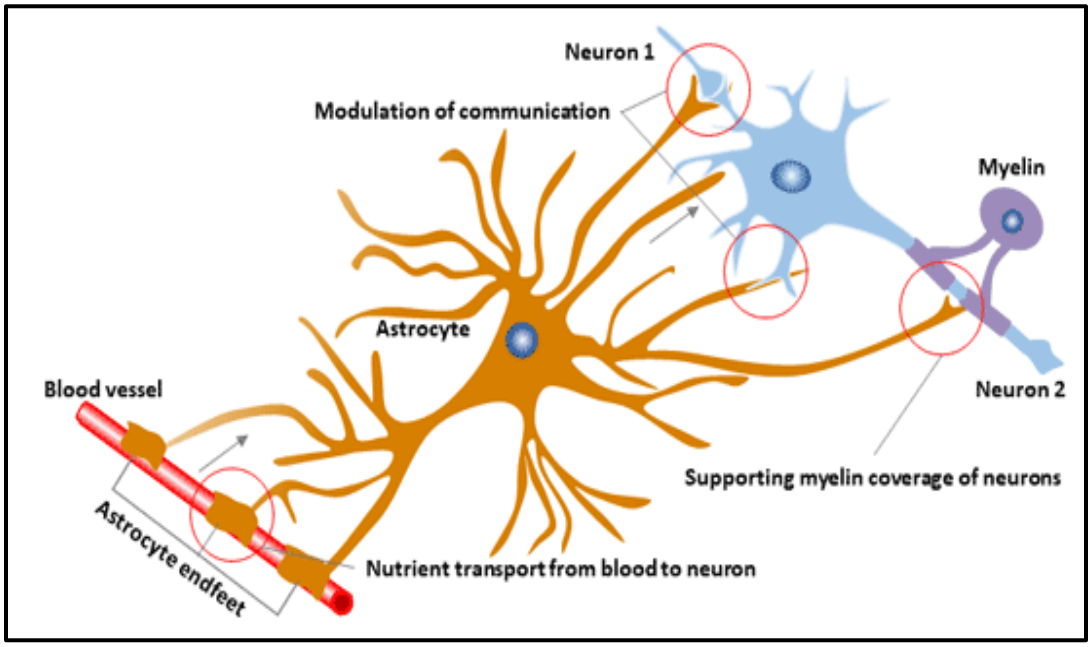

Figure: 1.5 Function of Astrocytes that includes numerous neural processes, including as synapse development including plasticity as well as energy which includes redox metabolism, as well as postsynaptic regulation of neurotransmitters and ions, are carried out by astrocytes.

Lawrence Eng announced the findings at an International Society of Neurochemistry gathering in September 1969. The central nervous system (CNS) marker GFAP was readily accepted, and its increased expression was later identified as a sign of gliosis correlated with brain damage as well

as illness (Brenner & Messing, 2021) . Our knowledge of GFAP's function in health and illness has steadily grown. With its influence now extending to genetic medicine and gene therapy, control of GFAP's expression and measurement of its levels are now pursued in both basic and clinical neuroscience (Booth et al., 2017).

1.6.1 Applications

A significant component of the glial intermediate filaments that make up the cytoskeleton of adult astrocytes is glial fibrillary acidic protein (GFAP). Nine GFAP splice variants have been identified in the human central nervous system as of this writing.

The neuroscience community soon accepted GFAP as a practical marker for cell identification since, as was already mentioned, it was primarily expressed by astrocytes of the central nervous system (Messing & Brenner, 2020). The importance of GFAP detection in tumor diagnostics swiftly became apparent after that. The presence of GFAP provides a highly useful tool for differentiating astrocytoma from other gliomas and tumors affecting the CNS, complementing earlier morphological evaluations that linked neoplastic cells in astrocytoma to their assumed normal counterparts (Kimura et al., 2019).

Although the presence of GFAP supported the astrocytic character of these cells, it has since proven challenging to link expression levels with the severity of malignancy. In order to subtype and grade CNS neoplasms, more precise immunohistochemistry testing, molecular and genetic phenotyping, and classic morphological characterization are increasingly being used. This new approach helps predict outcomes and direct therapy (Kimura et al., 2019).

Since the soluble phosphorylated pool of GFAP is in dynamic equilibrium with the protein's polymerized non-phosphorylated portion, the phosphorylation state of the protein regulates how the protein assembles. A strong control of GFAP during brain development and in the pathogenesis of numerous neurodegenerative diseases is supported by a number of lines of evidence (van Asperen et al., 2022). Alexander disease, a deadly neurological disorder, has been linked to GFAP gene abnormalities. Additionally, central nervous system abnormalities in GFAP expression and

phosphorylation have been repeatedly observed in cases of neurodegenerative diseases such Parkinson's disease (PD), frontotemporal dementia, and Alzheimer's disease (Imbriani et al., 2022).

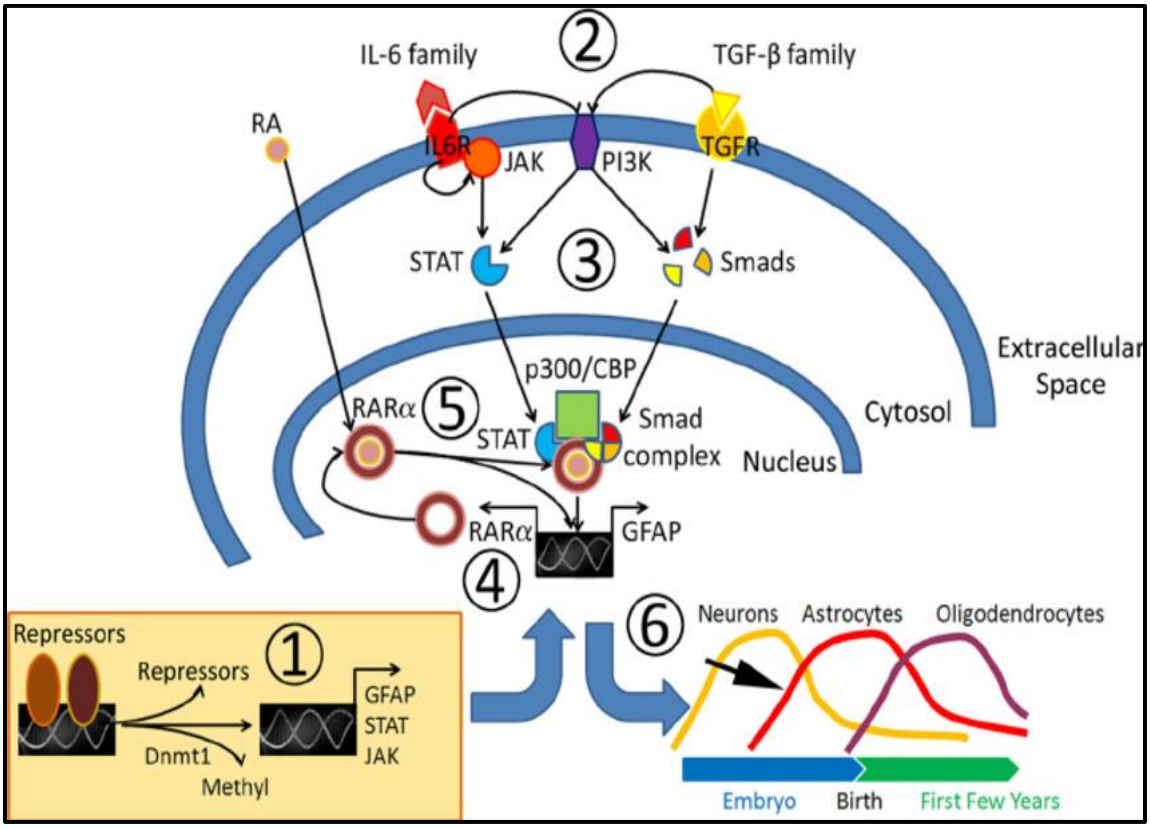

Figure 1.6 Different steps involved in applications of GFAP

The fact that numerous non astrocytic cells that resides both internal as well as external of the CNS produce measurable quantities of GFAP, in contrast to not all astrocytes, should limit the use of GFAP as a hallmark of astrocytes, specifically in its reactive state. Additionally, even while dye filling in the inside of cells depicts significantly additional complex architectonics of sheer processes than normal light microscopic immunostaining for GFAP, which is frequently employed to determine gross astrocyte morphology (Booth et al., 2017).

The appearance of GFAP in blood and cerebrospinal fluid (CSF) has drawn more attractiveness towards a diagnostic indicator of brain damage in addition to act like a hallmark of astrocytes. One may think of several methods, most notably cell death with subsequent release of typical cytoplasmic components, through which GFAP exits its intracellular position. Halford et al. (2017) could not clearly described that which regions of the protein these particles arise, but they suggested that the presence of 18- to 25-kDa degradation products in the CSF occur late after traumatic brain injury and imply cell death (Abdelhak et al., 2018).

Exosomes may also contain GFAP, and their release is actively regulated. GFAP levels in CSF as well as in the blood increases when there has been an injury or illness, often to startling levels. This is especially true in cases of traumatic brain injury, stroke, subarachnoid hemorrhage, and neuromyelitis Optica. Alexander disease, where GFAP is the illness's initiator and is likewise high, may benefit especially from observation of CSF as well as blood levels (Abdelhak et al., 2022).

CHAPTER TWO: LITERATURE REVIEW

2.1 Background

Parkinson's disease (PD) is the second most typical progressive neurological illness after Alzheimer's disease (AD) (PD). By the time they are 65 years old, about 3percent of the population has it, and by the time they are 85 years old, nearly 5percent of the population has it. Because of estrogen, men are roughly 1.5 times more likely than women to develop Parkinson's disease. Regarding the severity of the disease and how they react to various therapeutic therapy, patients exhibit a wide range of clinical phenotypes (Bonam & Muller, 2020).

In contrast to some non-motor symptoms like anosmia, sleep issues, GI disorders, anxiety and depression, PD patients also have some motor symptoms such bradykinesia, stiffness, tremors, and postural instability.

Although more research is still needed to understand the etiopathology of Parkinson's disease, most cases of the disease show multiple interrelated risk factors, such as genetics, environmental factors, and age (Rizzone et al., 2019). Degradation of mesencephalic dopamine neurons, which are found in the substantia nigra pars compacta, is a feature of PD inside the cell.

The presynaptic neuronal protein known as -synuclein/SNCA, which accumulates and aggregates in Cell bodies and Lewy neurites, is thought to be the primary cause of the majority of that kind of neurodegeneration in PD (Bonam & Muller, 2020).

Industrialization and the prevalence of PD have been regarded to have a close association as a result of the entrance of numerous chemicals into our household surroundings to combat the challenges of infestation, primarily due to the excessive utilization of pesticides in current contemporary times. To give two examples, the first organochlorine, DDT, was created in 1939 and the first organophosphate, triethyl-pyrophosphate, was recorded in 1854.

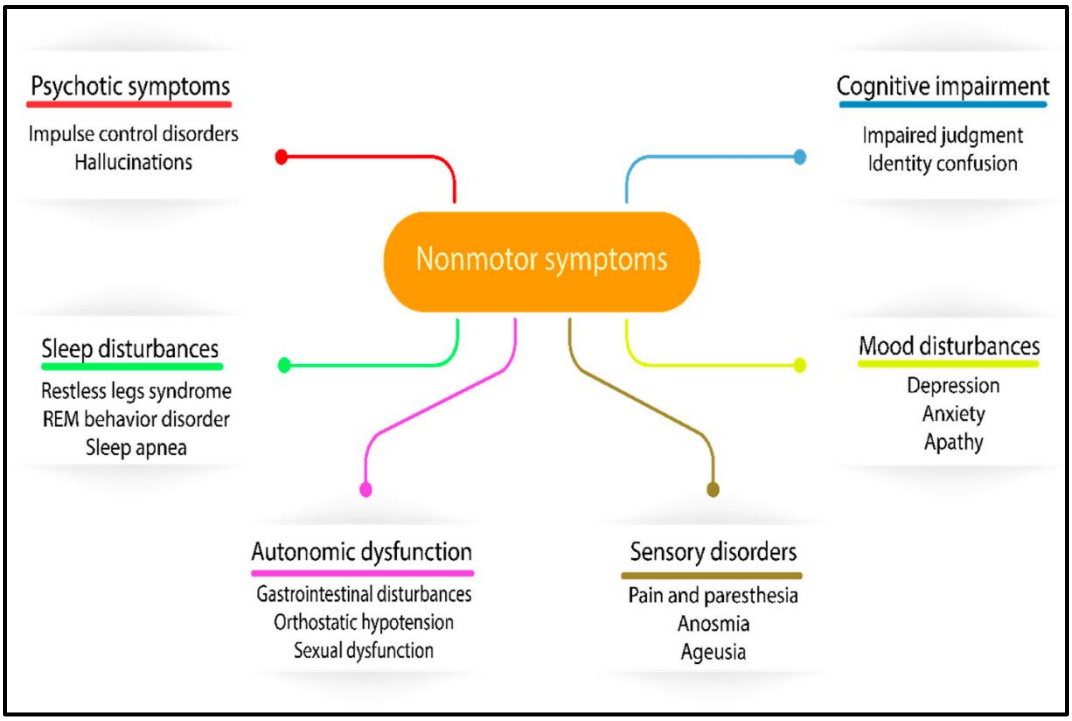

Figure: 2.1 A schematic diagram that shows non-motor symptoms of PD

However, pesticide use dates back to 1500 BC, when the Ebers Papyrus describes preparations for the eviction of fleas. Chinese garden pests were treated with arsenic as early as 900 AD. In the 1600s, nicotine was also utilized as an insecticide, and honey and arsenic were combined to make ant bait (Smith et al., 2020).

In the present era, aerial spraying or pesticide exposes thousands of pesticides to our food and water, contaminating the water, food, and other edibles. Despite historical evidence of acute pesticide poisoning, potential chronic toxicity, including such carcinogenesis with organochlorines and neuropsychiatric disorders with organophosphates, these connections between the development of PD and pesticide exposure have only recently been recognized and studied (Dorsey et al., 2018).

The recommended diagnostic for Parkinson's disease (PD) in Lewy bodies is cytoplasmic inclusion bodies positive for ubiquitin and harbouring numerous abnormal proteins. A gene mutation in the signaling cascade system called Ubiquitin ligase (PARKIN) contributes to Parkinson's disease

(PD), and when combined to Humoral immune response putative kinase 1 (PINK1), it makes it easier to get rid of damaged mitochondria (Bonam & Muller, 2020).

Tissues from post-PD patients also show compromised or disturbed proteosome and Lysome functioning. Lewy bodies are not developed in non-human primates, but the cellular pathways of clearance can be seen by the presence of Marinesco bodies, which are signaling cascade inclusions in the nuclei of neurons, and cytoplasmic lipofuscin accumulations, a byproduct of lysosome activity linked to the removal of damaged organelles like mitochondria.

These indicators show a stronger correlation between ageing and neuronal expression in the midbrain. Such Signaling cascade nuclear inclusions were discovered to be significantly and virtually solely growing with advancing age in the majority of susceptible vtSN neurons (Stefanis, 2012).

2.2 Mechanisms of MPTP toxicity

MPTP itself has no negative effects on the brain. MPP, the ultimate neurotoxin created via a 2 different biocatalytic process, is absolutely necessary. The lipophilic MPTP easily crosses the blood-brain barrier following systemic injection, whereas the charged MPP is unable to do so (le Heron et al., 2021). Monoamine oxidase B first converts MPTP to 1-methyl-4-phenyl-1,2-dihydroxypyridinium ion (MPDP) (MAO-B). It's interesting to note that dopaminergic neurons themselves do not contain MAO-B, which is mostly present in microglia and serotonergic neurons. In a later phase, MPDP spontaneously oxidises to MPP (Langston, 2017).

MPTP can theoretically be transformed by MAO-A or MAO-B. But MAO-B seems to be more likely to be the essential enzyme than MAO-A. Therefore, by blocking MPTP metabolism with MAO-B inhibitors as opposed to MAO-A inhibitors, MPTP neurotoxicity was successfully prevented. During therapy only with MAO-B blocker selegiline (deprenyl), there was a considerable drop in striatal MPP levels, highlighting once more how successfully MAO-B inhibition may limit MPP formation.

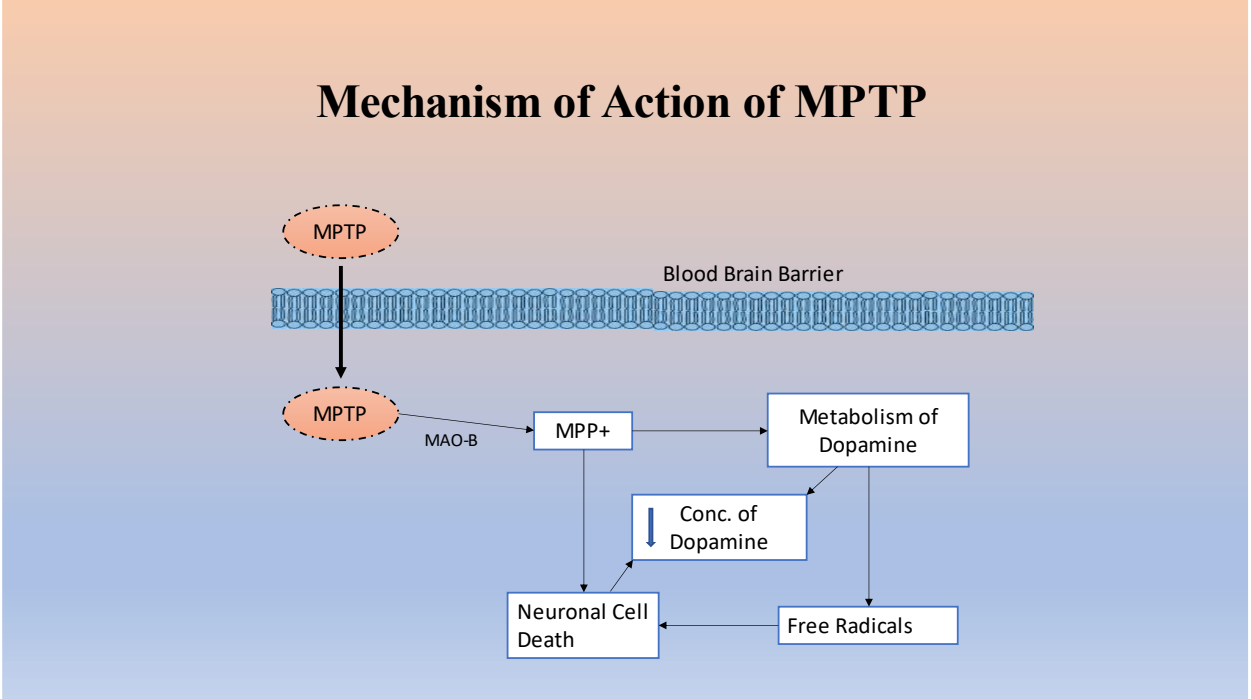

Figure: 2.2 Mechanism of Action of MPTP that includes the process in which blood brain barrier is easily crossed by lipophilic MPTP by following systemic injection, whereas the charged MPP is unable to do so. Monoamine oxidase B first converts MPTP to 1-methyl-4-phenyl-1,2-dihydroxypyridinium ion (MPDP) (MAO-B) and then to MPP+ which causes neuronal cell death that results in decrease conc. of dopamine. On the other hand, it causes damage to metabolism of dopamine. As a result of thus, conc. Of dopamine decreases as well as free radicals are produced.

Furthermore, animals with high MAO-B expression in the endothelial of central nervous system passageways of the blood-to-brain barrier, like rodents, are probably resistant to MPTP when MPTP penetration into the brains is hindered by rapid metabolism (Palencia et al., 2015).

Glial cells rapidly discharge MPP through into extracellular environment through the outer neurons in the brain monoamine transport mechanism, which was extensively studied by Russ and coworkers. Dopaminergic transporters (DAT) here on cellular membranes of dopamine nerve terminals can then preferentially take up MPP. Mice without the DAT are immune to MPTP poisoning, according to recent studies. Overexpression of DAT might make MPTP more neurotoxic (Venkateshappa et al., 2012).

The DAT is distributed on dendrites on both the terminal and cell body sides, indicating that MPTP poisoning may have an influence on both the brain and the soma.

MPP is taken up by vesicular monoamine carriers (VMATs) intraneuronally and stored in synaptosomal vesicles, or it aggregates in organelles by energy-driven absorption. MPP's cytotoxicity in a tube was decreased by intravascular storage by VMAT. By making MPTP more hazardous in mice deficient in the VMAT-2 gene, this was confirmed in vivo (Strafella et al., 2018).

2.2.1 MPTP Oxidative Stress Pathway

MPTP-induced neurotoxicity is exacerbated by oxidative stress. Because MPTP is converted to MPP+ by monoamine oxidase-B (MOAB) in glial cells, followed by DAT activity, MPP+ builds up in SNpc DAergic neurons. The mitochondria create reactive oxygen species (ROS), such as nitric oxide (NO), superoxide anion (O2), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH), as a result of this accumulation in DAergic neurons. When MPP+ is present, DA is delivered more quickly. As a consequence of increased auto-oxidation of both extracellular and intracellular DA, harmful phenolics and strong oxidizing •OH are generated (Gelders et al., 2018).

Excessive creation of •OH, which reacts at its site of synthesis in vivo, may be toxic to cells due to a number of chain events, including membrane lipid peroxidation, modifications in membrane permeability, protein inter, and DNA damage triggered by base pair mutations. Therefore, a contributing role in the degradation of DAergic neurons is actually increased •OH formation, which may overcome cellular antioxidant defence mechanisms (Marogianni et al., 2020).

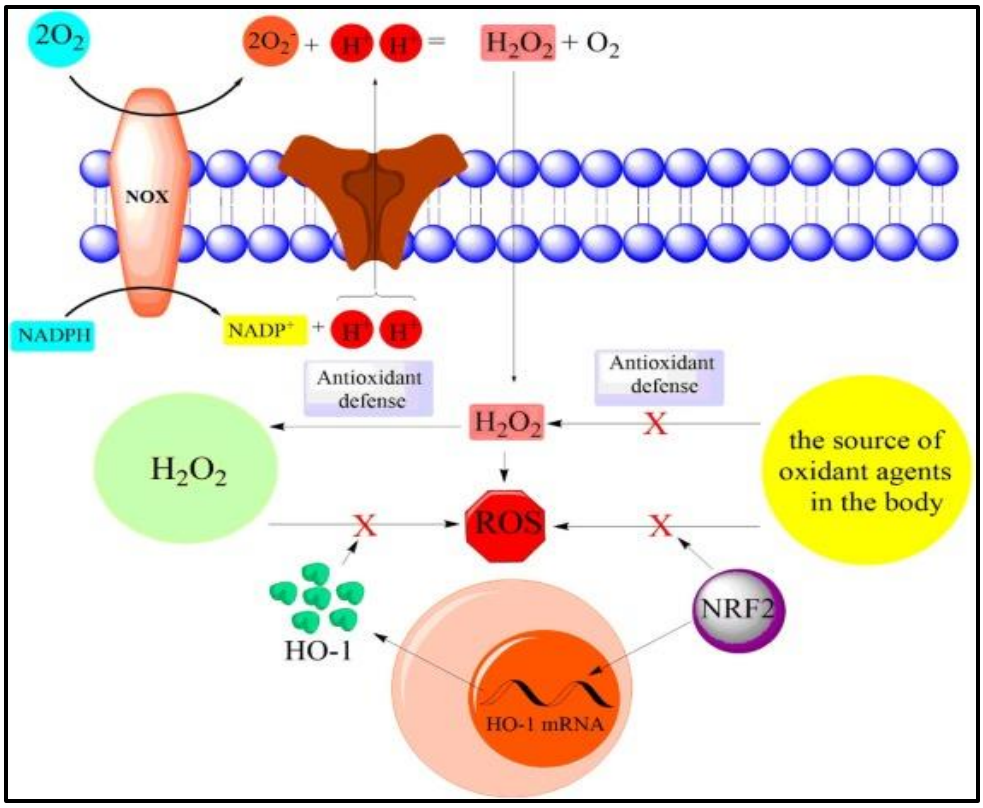

Figure: 2.3 Understanding how NOX and Nrf2 contribute to the OxS pathway. This is because NOX is a transmembrane enzyme that causes an increase in ROS production in the extracellular environment. Intracellular ROS delivery is facilitated by protein transporters. In cells, ROS are generated by several mechanisms and then contributed to the Redox pool. In response to an increase in intracellular reactive oxygen species (ROS), nuclear translocation of the transcription factor Nrf2 causes an increase in the expression of genes such heme oxygenase-1 (HO-1).

Some theories contend that the mitochondrial apoptotic cascade plays a major role in the MPTP-induced DAergic neurotoxicity. The increase of cytochrome c & caspase-9 in the striatum after MPTP therapy lends credence to this idea (Mestre et al., 2021).

The apoptotic cascade in mitochondria is mediated by these enzymes. Additionally, overexpression of the neuronally produced proteins Bcl-2, an anti-apoptotic protein, and p35, a caspase inhibitor, offered protection from the neurotoxicity brought on by MPTP. The mitochondrial apoptotic mechanism requires both the release of cytochrome C from the mitochondria and the activation of the mitochondria transition pore. Significantly, MPP+ blocks

complex I and creates Radicals to induce the activation of the mitochondria transition pore (Emamzadeh & Surguchov, 2018).

Cytochrome c is produced, and then it joins with pro-caspase-9 and apoptotic proteolytic activating factor 1 to form a complex that activates caspase-9 and then downstream caspases. The activation of the signaling pathways molecule knuckling is required for the release of cytochrome c, the expression of apoptotic protease activating factor 1, and the induction of caspase-9 after an event that promotes apoptosis. MPTP medication prevented the cytochrome c release from being released and reduced the mortality of DAergic neuronal tissue in the SNpc in null mice (Hamadjida et al., 2019).

2.2.2 Excitotoxicity Pathway

Data suggest that MPTP-induced DAergic neurodegeneration involves excitotoxicity. In this scenario, the blockage of complexes I of the electron carriers cycle in the mitochondria causes cellular ATP to be depleted, that various capacities the membrane permeability of SNpc neurons and increases external glutamine concentrations, which in turn triggers NMDA synapses on DAergic neurons (Fleisher et al., 2020) . Extracellular glutamate was discovered to have tripled in vivo utilizing micro dialysis after receiving prolonged MPTP treatment. The affinity of the SNpc glutamine transporters for glutamate is also increased by the toxin treatment. The glutamate transporters in the SNpc had a higher affinity for glutamate after receiving the toxin treatment (Binvignat & Olloquequi, 2020).

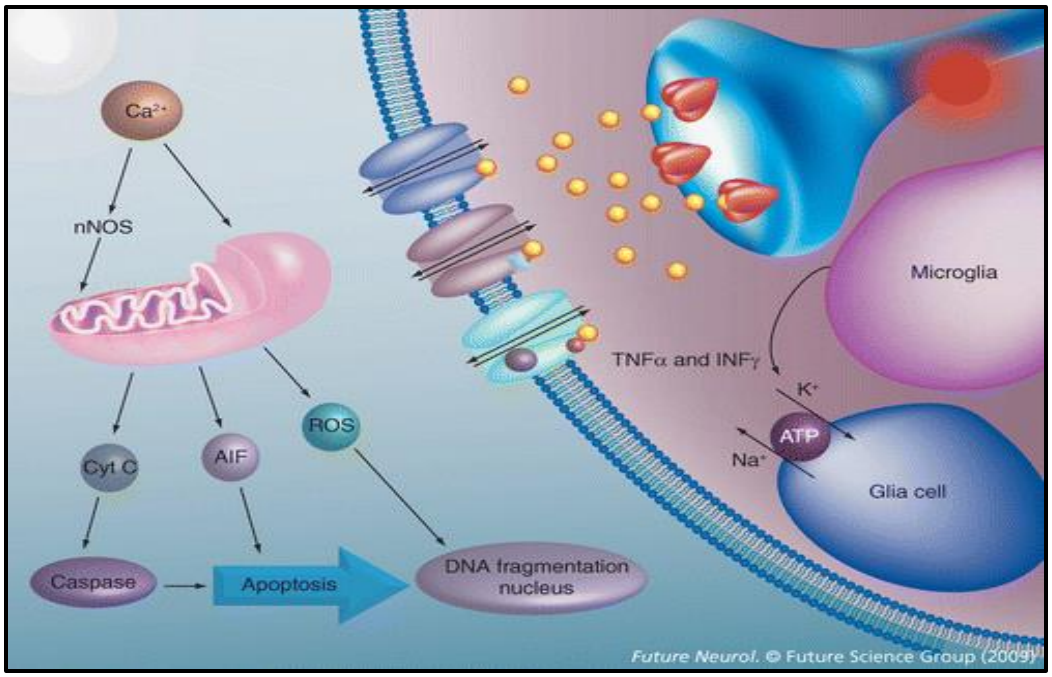

Figure: 2.4 Function of glial cells

Even though the glutamatergic supplies causing these elevated levels are not known, they might include near the area glia, enhanced cortex or subthalamic discharge from the axon terminals of DAergic neurons, and/or a sharing with the receptor activation cotransporter, which transfers receptor activation from the mitochondria of nerve endings even after seeming to be calcium (Ca2+) insensitive. The second theory, however, is still up for debate (Iovino et al., 2020)

2.3 Vulnerability of different strains

It has been established that MPTP is neurotoxic to humans, other primates, animals, rabbit, and some rodents. Only some mouse strains are sensitive to MPTP in mice, indicating that genetics may play a role in how MPTP works. There are differences across mice strains in the degree of ventral striatum DA deplete, the loss of striatum DA neurons, and behavioural deficits in response to MPTP. According to the percentage of lost SNpc neurons, different mouse strain can be categorised as "sensitive" (i.e., >50percentage SNpc lost) or "resistance" (i.e., 25% SNpc loss). The reasons for the variations in MPTP susceptibility amongst mouse strains are unknown (A Anandhan, 2017).

The phenomena has been the basis of several hypotheses, yet none of them fully explain it. The enzyme MAO-B catalyzes the reduction of such MPTP protoxin to the dyhdropyrididinium intermediate 1-methyl-4-phenyl2,3-dihydropyridinium species (MPDP+), that is ultimately oxidised to the lethal MPP+, and its activity affects the neurotoxicity of MPTP (Darweesh et al., 2018a). It has been proposed that shifts in brain MAO-B activity could account for differences in genus and strain vulnerability to MPTP. It has been proposed that differences in MAO activity are the reason why MPTP does not make rats toxic (SS Ahmed, 2009).

The only mice strains that demonstrated that brains MAOB activity was greater than hepatic MAOB activity were the C57BL/6 and Bulb/C strains, which are the species most susceptible to MPTP. Therefore, this mouse strain's heightened susceptibility to MPTP may be caused by the liver MAO-restricted, B's systemic detoxification of MPTP (Zahoor et al., 2018a).

Some other possibility for the variability in vulnerability to MPTP across different strains is that they have different thresholds for oxidative changes. Although free radical generation is believed to play a substantial role in MPTP-induced cell death, little is known about the different oxidation states of distinct mouse strains, particularly the SNpc (Bhurtel et al., 2019b).

The Swiss-Weber strain, which is resistant to MPTP, and the C57BL/6 and Bulb/C strains, which are susceptible to MPTP, did not differ in their ability to produce reactive oxygen species in the striatum, suggesting that the free radical production by itself is insufficient to account for the variability in strain susceptibility (de Bie et al., 2020).

Diverse DAT uptake, divergent DAT kinetics, divergent glutamate transporter function in astrocytes, divergent regulatory oversight of Calcium ions+ flow into SN neurons, and divergent properly functioning variability in the electron transportation chain proteins have also been investigated as additional possible reasons for discrepancy sensitivity to MPTP. Sadly, none of them can pinpoint the specific contributions of these research to MPTP-induced neurotoxicity (Abhilash et al., 2021).

According to a theory, different mouse strains have different levels of sensitivity, with pigmentation variants being much more susceptible than albino ones. Behavioral impairments, DA deplete in the stria, and neuron death in the SNpc brought on by MPTP were not prevented in mice with a minor mutation in the gene responsible for tyrosine hydroxylase, the enzyme that catalyses the first two steps of pigment formation, located on chromosome 7. Melanin pigments are inadequate, which causes albinism. If a susceptibility gene is present within the same chromosomal area as the greater MPTP sensitivity observed in pigmented mice, further investigation is required to confirm this (Pathania et al., 2021).

2.4 Pros And Cons Of MPTP Mice Models

It is fundamentally assumed that the MPTP mouse model adequately simulates naturally occurring neurodegeneration. It is without a doubt true that MPP+ is a potent complex I inhibitor inside the midbrains of mice and PD patients. The decrease of Dopamine in the striatum is also a result of the axonal degradation and death of DAergic neurons (Baggio et al., 2015). Therefore, ventral striatum DA depletion should indicate the demise of SN DAergic cells, just like it does in PD patients.

In addition, the topographical distribution of DAergic loss of neurons in PD patients and the mouse midbrain is comparable. In other words, neuronal loss is concentrated on the lateral layer and laterally SNpc neuron as well as the posterior locations, bypassing more the anterior and medial cells (Centner, 2021). But it's still not clear if it precisely mimics PD. The mouse model of Parkinson's disease may be used to study mitochondrial malfunction.

The model should take into account the gradual progression and behavioural features of Parkinson's disease (PD), which is a neurodegenerative disease like other neurodegenerative diseases. The reduction of substantia nigra DA does not considerably advance with Rademacher/Rodents MPTP Modeling MPTP treatments, although DAergic neurons quickly die (Pasquini et al., 2018). One of the most advanced MPTP treatments involves the continued delivery of the neurotoxin over a number of weeks since neuron continue to die long after the poison has ceased being given. With the majority of MPTP administration modalities, the pattern of DAergic

terminal loss in the striatum does appear to match that of PD. In addition, extra-nigral disease has been documented in lower levels of monoamines other than DA (Klemann et al., 2017).

Table 1
Mouse model features that recapitulate PD. General definitions of the models appear below*

Toxin	Time to greatest DA cell loss	Striatal DA loss	DAergic cell loss in SN	Inclusions
Single dose MPTP (30 mg/kg in mice)	12 hours	>80% loss of DA after 1 day and > 40% loss after 30 days	20–30%	Not examined
MPTP, acute* (mice)	12 hours	>90% loss of DA 7 days after treatment	40%	None
MPTP, subchronic (subacute)* in mice	12 hours	53% loss of DA 30 days after treatment	24–40%	None
MPTP/P, chronic* in mice	3 weeks post-treatment	95–98% loss of DA 3 weeks after treatment; 76% loss after 6 months	50% post-treatment and 70% 3 weeks post-treatment	Proteinaceous and lipid inclusions in secondary lysosomes that are α-synuclein-positive but do not resemble Lewy bodies
MPTP, chronic* with escalating doses in mice	At the end of treatment (4 weeks)	Dose-dependent loss of DA; greater than 70% loss of DA with the two highest doses only	24% loss after 1 week and 62% after 4 weeks following the highest dose	Not examined
MPTP, chronic* with mini-pumps in mice	21 days	85% loss of DA metabolites in dorsal striatum	75–80%	α-synuclein-positive inclusions that do not resemble Lewy bodies inclusions
MPP+, chronic pump in rats	42 days or longer	Dose-dependent loss of DA	40%	No inclusions in DA neurons

Table 1: Different regimes of MPTP administration

The toxin was initially administered in a regimen involving a series of infusions either of 10 mg/kg or 20 mg/kg over the duration of a day at intervals of at least an hour. Tyrosine hydroxylase (TH) reactivity demonstrates that MPTP can kill DAergic neurons for a prolonged period of time after killing them initially. It is well known that now the scheduling of MPTP treatments in mice can result in wildly different results. Injections of MPTP are frequently given intravenous (i.v.) or subcutaneously (s.c.); i.v. No longer used are injections (Bourque et al., 2016).

This acute regimen results in a 40–50% loss of midbrain DAergic neurons. The survived DAergic neurons have not been shown to have inclusion bodies, and the neurons don't seem to die naturally

by apoptosis or any other process. However, mouse mortality might vary from 50% to higher (Darweesh et al., 2018).

If the dose is spread out over several days, the toxin appears to be cleared more efficiently (within the initial 3–12 hours) and the SN of mice do not lose as many DAergic cells (once or twice daily injections over 5–10 days; sub chronic or subacute regimen). So, when poison is injected into a patient using a minipump over the course of 28 days (Ryan et al., 2015).

2.5 OBJECTIVE

- Parkinson's disease remains an unsolved clinical problem, as currently authorized PD therapies offer relatively modest therapeutic benefits.
- New therapeutic approaches that not only alleviate symptoms in the short term but also stop the disease from getting worse are desperately needed.
- The main objective is functional characterization of proteomic factor GFAP as a risk factor in an effort to enhance the efficacy of the treatment of PD in mice models.

CHAPTER THREE: METHODOLOGY

3.1 Outline of Materials and Methods

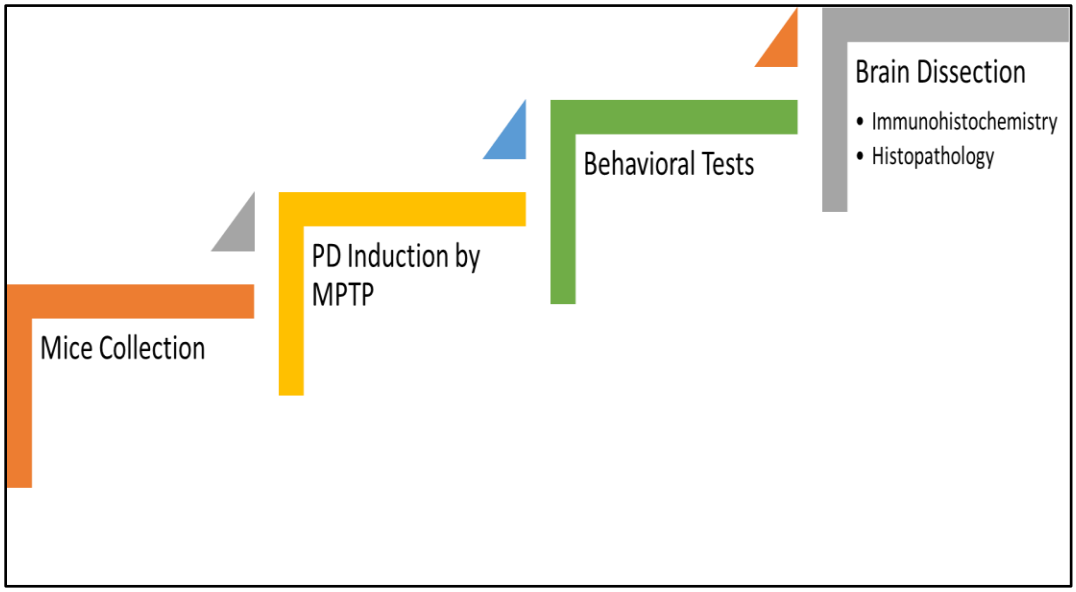

Figure:3.1 Methodology of research

3.2 Materials

The materials used for this study are as follows:

3.2.1 Reagents

Mice, Sterile saline, MPTP. HCl powder, Commercial bleach, Paraformaldehyde powder, Sodium phosphate buffer.

3.2.2 Equipments

A procedure room with a fume hood and sink that has been authorized by the institution's animal care and use committee (temperature range: 22–27°C). A stationary safe-box to house the MPTP supplies. Powder HCl covering material with a non-absorbent side and an absorbent side. Absorbent sheets with a plastic backing to cover the whole floor of the operation room. Protective equipment mouse-weighing scale MPTP microbalance for weighing. For the injections, disposable plastic tuberculin syringes with 27-gauge needles are used. Sterilized, disposable glass tubes (14 ml, 30 ml). Permanent markers tidy mouse pens. Magnification device. Dry ice is used to freeze tissues after dissection and wet ice for dissection. Using aluminum foil to wrap and store tissues centrifuge that is chilled. a -80 1C freezer for sample storage connected light microscope.

3.2.3 Reagent Setup

Male adult BALB/C mice weighing between 25 and 28 g were utilized for MPTP investigations. To generate a repeatable lesion, chosen mice were 8 weeks old. All animal-related research was conducted in accordance with institutional and national guidelines.

3.2.4 Equipment setup

The fume hood and the animal rack, which houses mouse cages and other cages, were both covered with covering material.

3.3 Experimental Design

- Total of 20 male mice were taken in this study.
- In the initial experiment, mice were split into two groups of ten each.
- The other group of ten mice got a dosage of 20 mg/kg MPTP, whereas the first group of ten mice served as the control (i.p.).
- Tremor was observed visually for 45 minutes following the second MPTP injection.
- Three hours after the second MPTP injection, akinesia and catalepsy were assessed.
- Swim tests were performed on the fourth day after the first injection.
- Open field testing took place on the sixth day.

- A rearing test was carried out on the sixth day.
- Mice were again weighed on 8th day before dissections.
- Animals were dissected on day eight, and formalin-fixed brain tissue samples were obtained. 2
- Then, using immunohistochemistry and various staining techniques, such as Hematoxylin and Eosin (H &E) and GFAP staining, the DA levels in the excised striatum and substantia nigra pars compacta were examined.

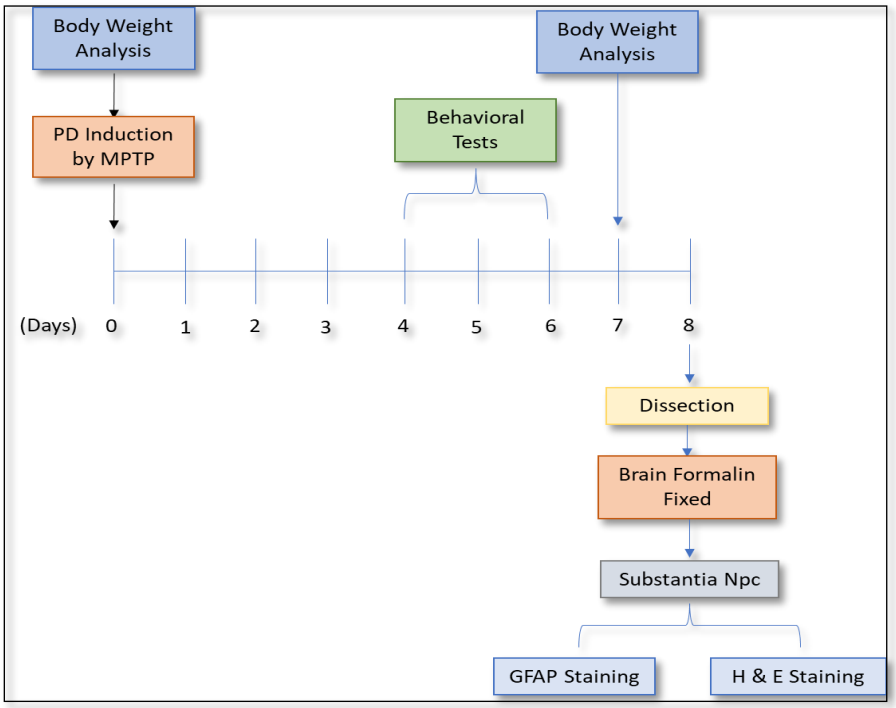

Figure: 3.2 Procedure of this study that includes mice were weighed before MPTP administration. Swim tests were performed on the fourth day after the first injection. Open field testing took place on the sixth day. A rearing test was carried out on the sixth day. Mice were again weighed on 8th day before dissections. Animals were dissected on day eight, and formalin-fixed brain tissue samples were obtained.

3.4 Methods

3.4.1 Housing and Acclimation

Mice were obtained, housed in a controlled environment (22–27°C) for a week prior to injections, and given time to adapt. Mice were housed in separate cages with access to food and water in a 12-hour light/dark cycle. The standard of good laboratory practice established by the US FDA (Food and Drug Administration) in 1978 served as the foundation for handling and caring for mice.

3.4.2 MPTP administration

Mice were weighed, sorted, and coded the day before MPTP treatment began. One cage contained five mice. The mice's tails were marked with permanent markers to help with identification. Throughout the operation, normal or control mice were maintained in cages without receiving any toxic treatment, whereas diseased animals received intraperitoneal injections of neurotoxic. An acute dosage of 20 mg/kg free base MPTP was delivered intraperitoneally (i.p.) to male mice aged 8 weeks, and toxicity tests were performed on day 8. MPTP was combined with saline and administered intraperitoneally at dosages of 20 mg/kg every two hours for a total of four times throughout an eight-hour period in one day. After the final MPTP injection, mice were allowed to rest in the restricted procedure room.

Figure 3.3: MPTP administration

3.5 Behavioral Tests

3.5.1 Swim Test

On the fourth day following MPTP administration, a swim test was conducted in water tubs that were 40 cm long by 25 cm wide by 16 cm high. The water was held at a depth of 12 cm, and the temperature was fixed at 27 2 C. As was intended by the test's design, mice tried to escape but were unsuccessful. Animals were taken from the water tub as soon as they gave up after a specified amount of time and became immobile. Following the trial, the animals were dried down with a dry towel before being put back in cages that were kept at a constant 27 2 C.

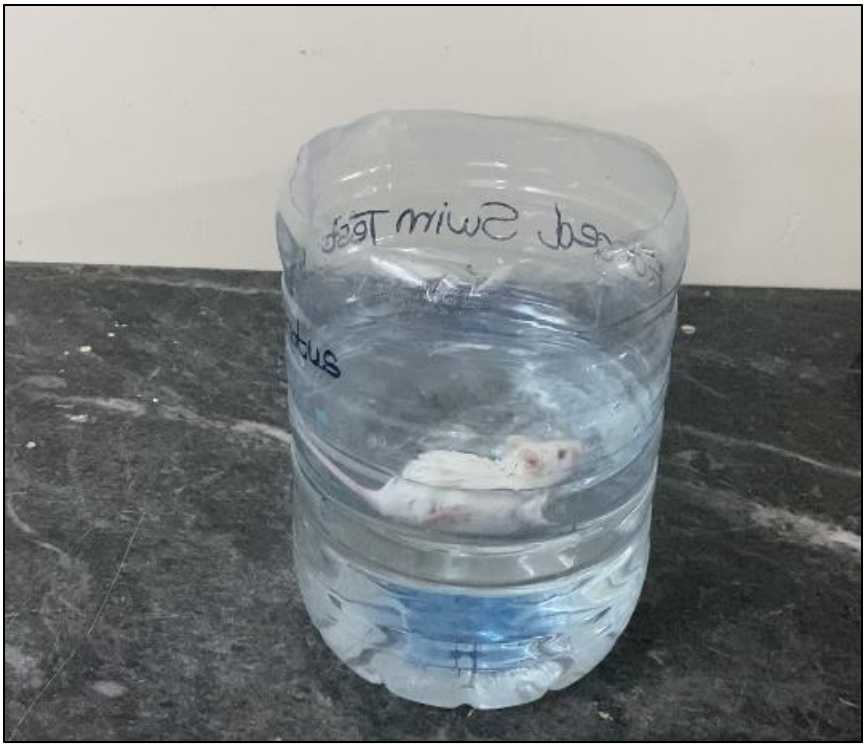

Figure 3.4: Visual representation of Swim test

3.5.2 Open field Test

In mouse models of CNS diseases, the open field test is used to measure general activity levels, gross locomotor activity, and exploratory behaviors. For this exam, evaluation occurred in a square with a margin line dividing the box's center from its edges. Mice were confined in a square cage, and their activity was monitored for five minutes. The mice were then put back in their cages.

Figure 3.5: Visual representation of Open field test

3.5.3 Cylindrical rearing test

To assess locomotor asymmetry in mouse models of CNS diseases, the Cylinder test was developed. A mouse travelled inside an open-top, transparent plastic cylinder, and the activity of its forelimbs as it reared up against the arena wall was recorded.

The full palm was placed on the arena wall, indicating that the forelimb was being used to support the body. As a fraction of all contacts, the number of impaired and unimpaired forelimb contacts was computed.

Figure 3.6: Visual representation of Cylindrical rearing test

3.6 **Mice Body weight**

On 8th day before dissections, mice were weighed to analyze the impact of Parkinson's disease on body weight in this experiment.

3.6.1 Statistical analysis

The neurochemical tests were utilized to identify significant differences between two means using the Student's t-test. Graph Pad Prism software was used for behavioral testing (swim test, central open field test, peripheral open field, rearing test) using the statistical package. The data for behavioral investigations were statistically analyzed for significance using non-parametric analyses. It was done to analyses correlations. The results are displayed as mean S.E.M. values. Significant values were those with ≤ p 0.05.

3.7 Dissection of brain regions

Mice were anesthetizing with chloroform. A pair of sharp scissors was used to decapitate the mouse. Both hemispheres were separated by using a scalpel to cut down the middle of section of the brain. Striatum and Substintia nigra pars compacta tissues were collected and fixed for dopaminergic neurons analysis by using different staining.

3.8 Fixative

For Hematoxylin and Eosin (H &E) staining, brain tissue samples were fixed in a 10% formalin solution.

For GFAP staining, brain tissue samples were fixed in a 10% formalin solution.

3.9 Fixation Procedure

Use 60 ml yellow-capped containers with 30–40 ml solution volumes or 20 ml scintillation vials with 15-20 ml solution volumes to fix, wash, and dehydrate the samples.

For H&E staining, well-fixed samples can be kept in the same fixative for a few days or even longer at 4 C. After an overnight fixation, immunohistochemistry samples need to be dried and kept at -20 oC with 100% ethanol.

3.9.1 Tissue Fixation

After dissection, immediately brain tissue was dipped in the cold PBS. To remove all the blood from the sample, PBS was used to wash it off from the tissue. 10% formalin fixative was used to fix the tissue by placing the tissue in fixative solution.

CHAPTER FOUR: RESULTS

4.1 Impact of PD on body weight

A significant effect of PD on body weight of mice were observed. Body weight of mice which belonged to diseased group gradually decreased after MPTP administration whereas the body weight of mice which belonged to control group was normally increased with the trend of age. This is shown in graph

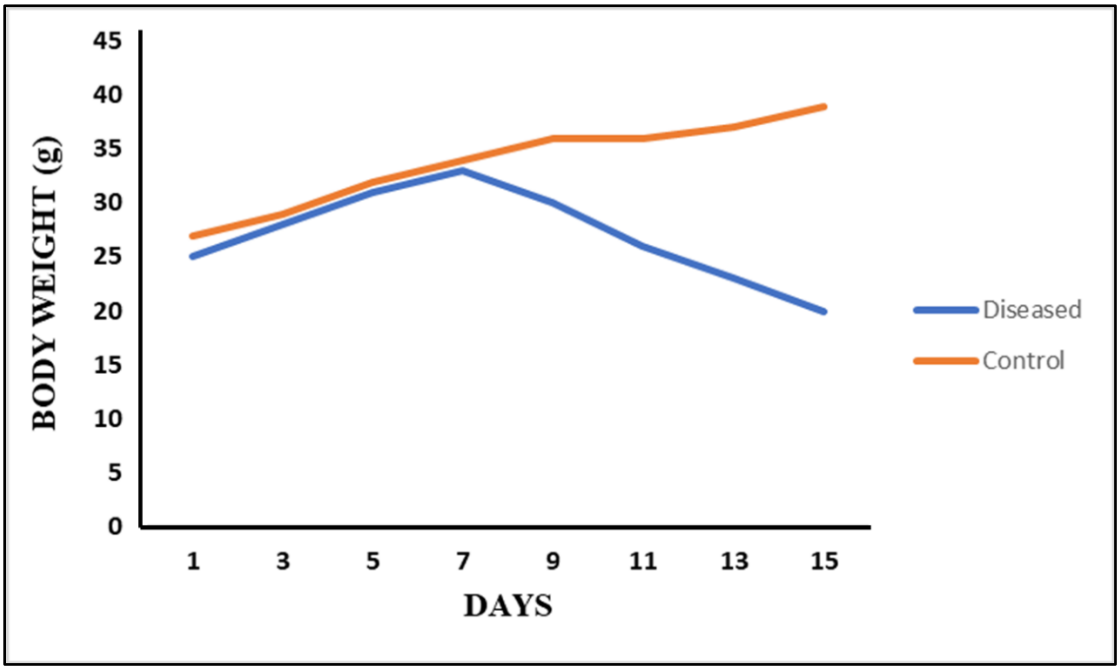

Figure 4.1: Body weights of animal model

4.2 Swim Test

The results of immobility and motor impairment of swim test depends on the time on which the mice show immobility as mice shows immobility after a certain period. A significant increased time period of control group was observed at which point they showed immobility as compared to the diseased group. It confirms less immobility and less motor impairment in control group as compared to the diseased group whereas more immobility and motor impairment was observed in diseased group as represented by the p value <0.0001.

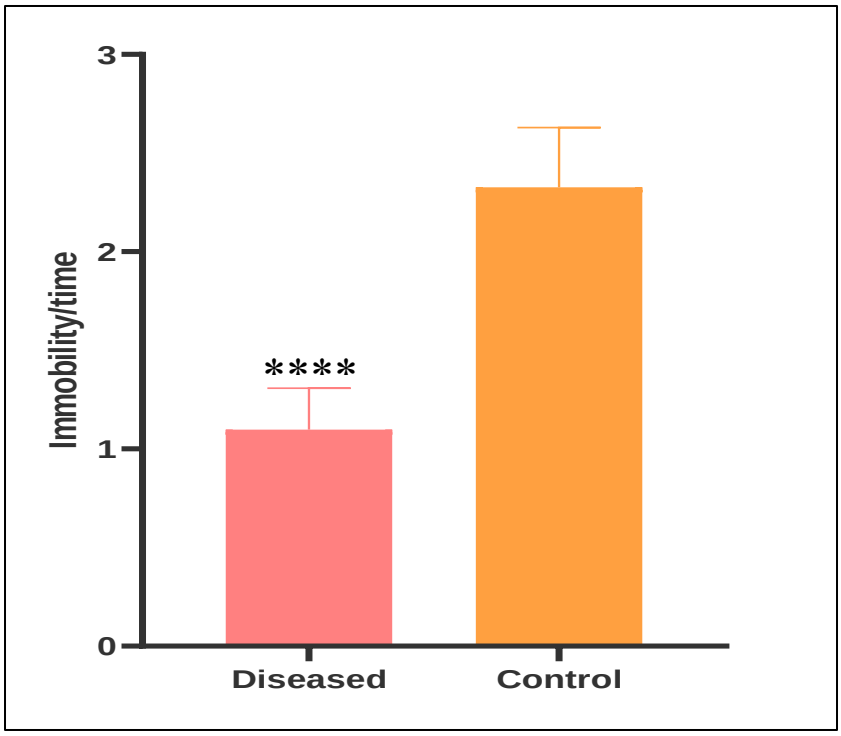

Figure 4.3: Graphical representation of Swim Test

4.3 Open Field Test

In the open field test, both mouse groups engaged in autonomous exploratory behavior and activity. Mice in the normal or control group were more active than those in the sick group in terms of total distance travelled ($P < 0.001$).

4.3.1 Central Open Field Test

The results of anxiety level and exploratory activity level depends on the visits of mice from central area to peripheral area and vice versa. A significantly increased number of crossing the margin line from peripheral area to centre area was observed in control group as compared to diseased group. It confirms reduced anxiety level and increased exploratory activity level in control group

whereas increased anxiety and exploratory activity level was reduced in diseased group as represented by the p value <0.0001.

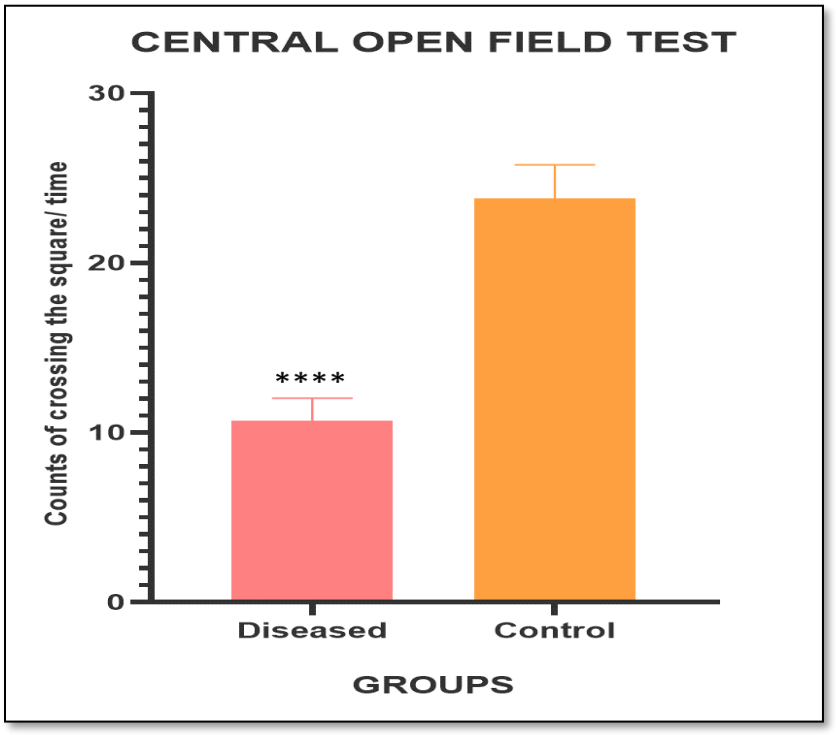

Figure 4.4: Graphical representation of Central Open Field Test

4.3.2 Peripheral Open Field Test

The results of anxiety level and exploratory activity level depends on the visits of mice from central area to peripheral area and vice versa. A significantly increased number of crossing the margin line from center to peripheral area was observed in control group as compared to diseased group. It confirms reduced anxiety level and increased exploratory activity level in control group whereas increased anxiety and exploratory activity level was reduced in diseased group as represented by the p value< 0.0001.

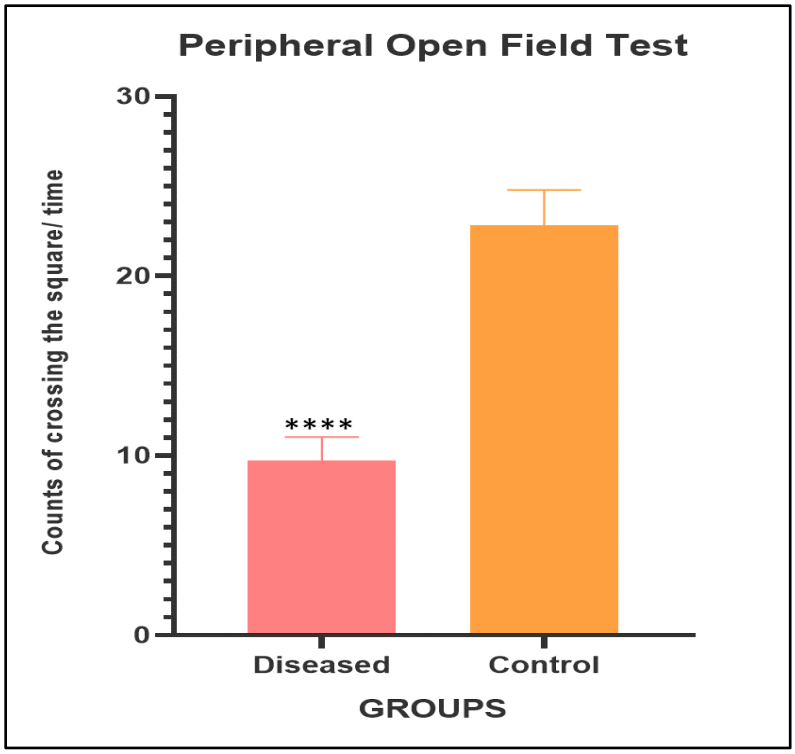

Figure 4.5: Graphical representation of Central Open Field Test

4.4 Cylindrical Rearing Test

The results of motor impairment and anxiety level of cylinder rearing test depends on the number of attempts of mice each time rears up against the walls of the cylinder by standing on the hindlimbs as well as the mice paw touches the wall of the cylinder. A significantly increased number of attempts of mice against the wall of the cylinder was observed in control group as compared to the diseased group. It confirms reduced anxiety level as well as reduced motor impairment and increased exploratory activity level in control group whereas increased anxiety level as well as increased motor impairment and exploratory activity level was reduced in diseased group as represented by the p value <0.0001.

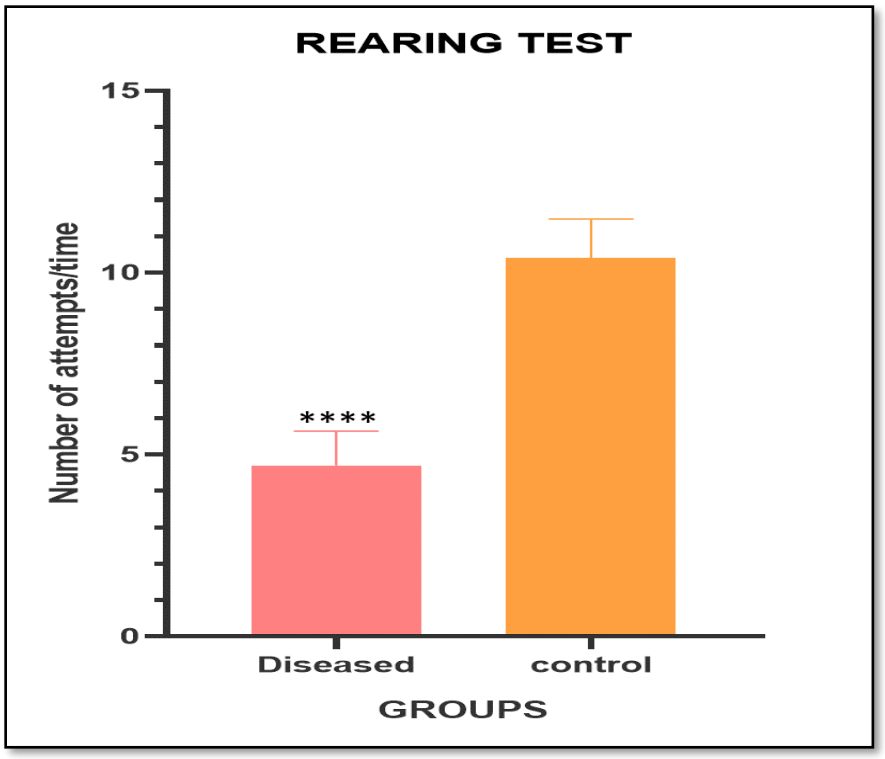

Figure 4.6: Graphical representation of Cylindrical Rearing Test

4.5 Hematoxylin and Eosin (H &E) staining

H & E staining of brain tissues specifically substantia nigra pars compacta demonstrated the pattern, shape and the structure of cells in that specific area which is often used to diagnose the disease. Hematoxylin stains nucleus of this tissue sample which is represented in blue color. Eosin stains extracellular cytoplasmic area which is represented in pink color.

Different morphology is shown after these staining which represents a clear difference in nucleus of cells, pattern and shape of the cells in both control and diseased group. H & E staining of control group showed normal nucleus as well as cytoplasmic area in normal pattern, shape and structure of cells. No neuron loss is noted.

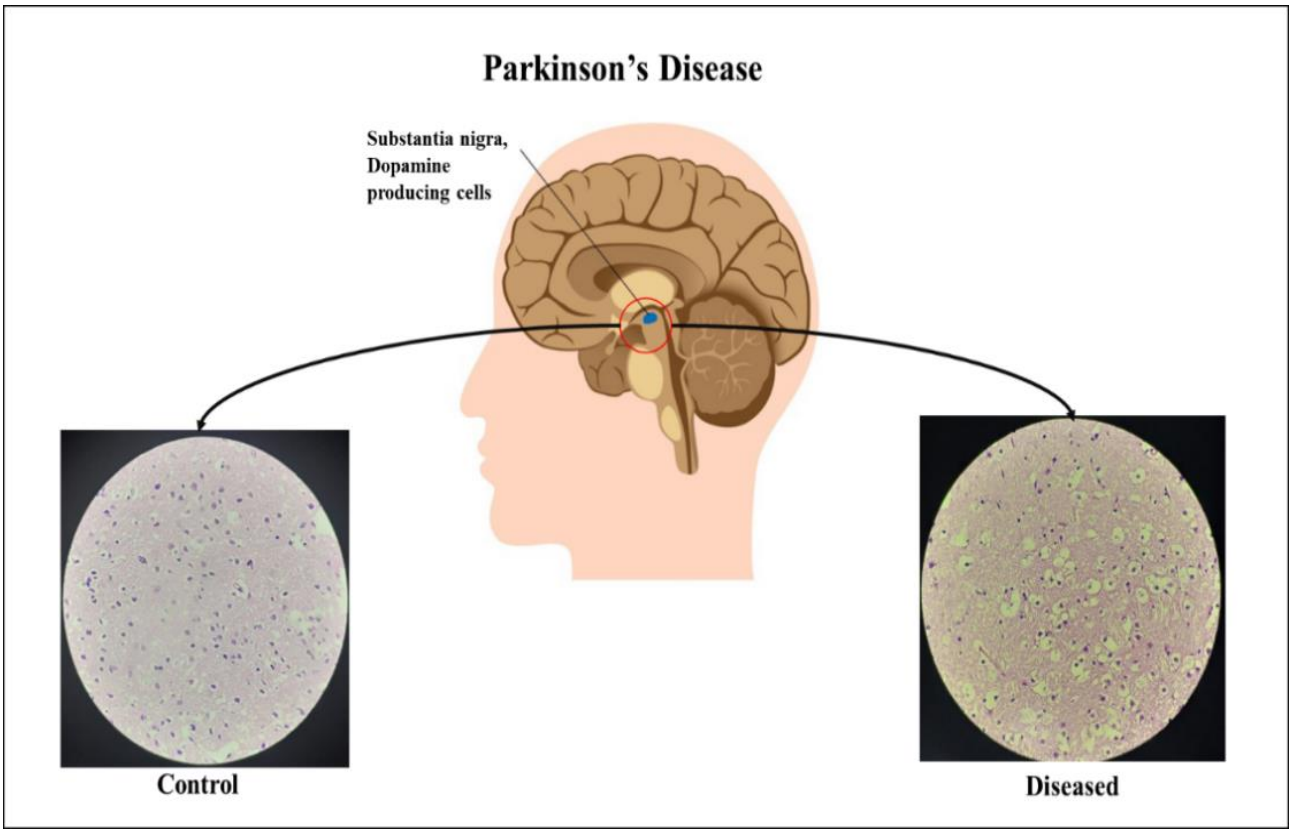

Figure 4.7: Visualization of SNpc H&E Staining H & E staining of control group show*n* in left side of fig showed normal nucleus as well as cytoplasmic area in normal pattern, shape and structure of cells. No neuron loss is noted. In contrast, H & E staining of diseased group showed altered morphology of nucleus and cytoplasmic area than normal which demonstrates the disease. It showed cluster of foamy histiocytes. Loss of neurons was observed in this region.

In contrast, H & E staining of diseased group showed altered morphology of nucleus and cytoplasmic area than normal which demonstrates the disease. It showed cluster of foamy histiocytes. Loss of neurons was observed in this region.

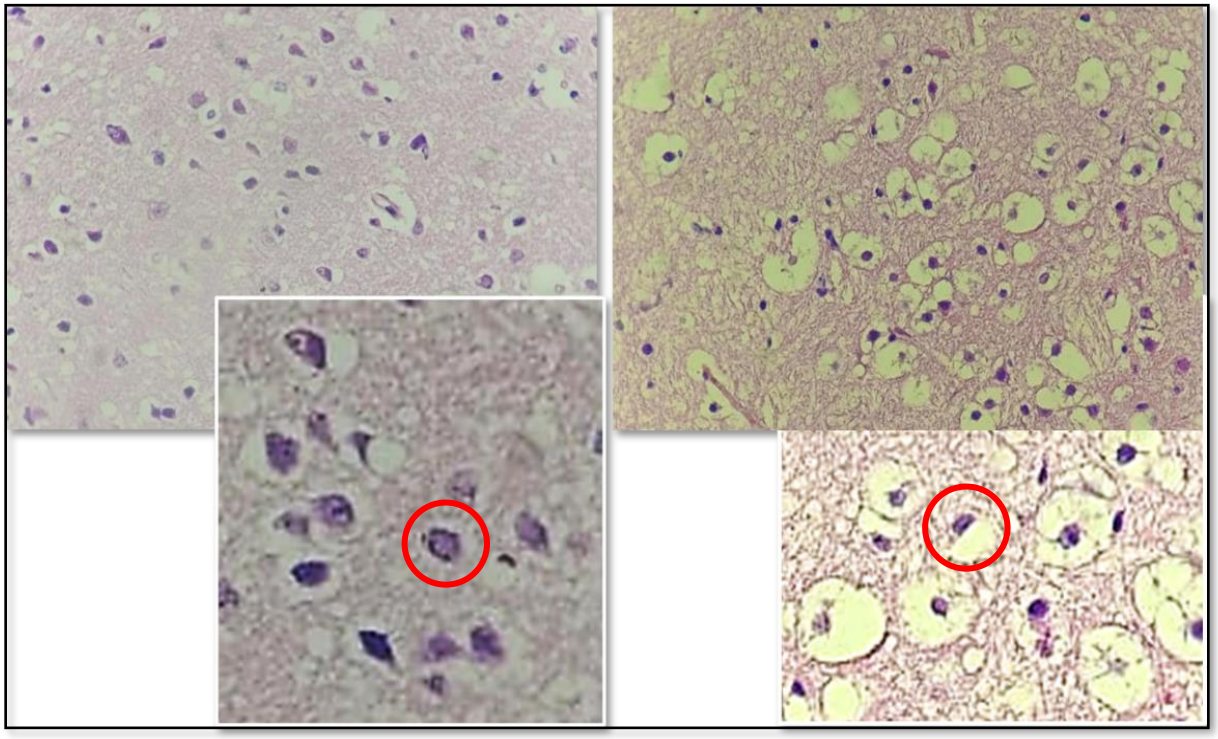

Figure 4.8: Insight visualization of SNpc H&E Staining H & E staining of control group show*n* in left side of fig showed normal nucleus as well as cytoplasmic area in normal pattern, shape and structure of cells. No neuron loss is noted. In contrast, H & E staining of diseased group showed altered morphology of nucleus and cytoplasmic area than normal which demonstrates the disease. It showed cluster of foamy histiocytes. Loss of neurons was observed in this region.

4.6 GFAP Immunohistochemistry

GFAP is a Glial Fibrillary Acidic Protein. It is located in astrocytes. Differentiation as well as astrological origin is evident by the expression of GFAP. Neural damage correlate with increased GFAP immunoreactivity.

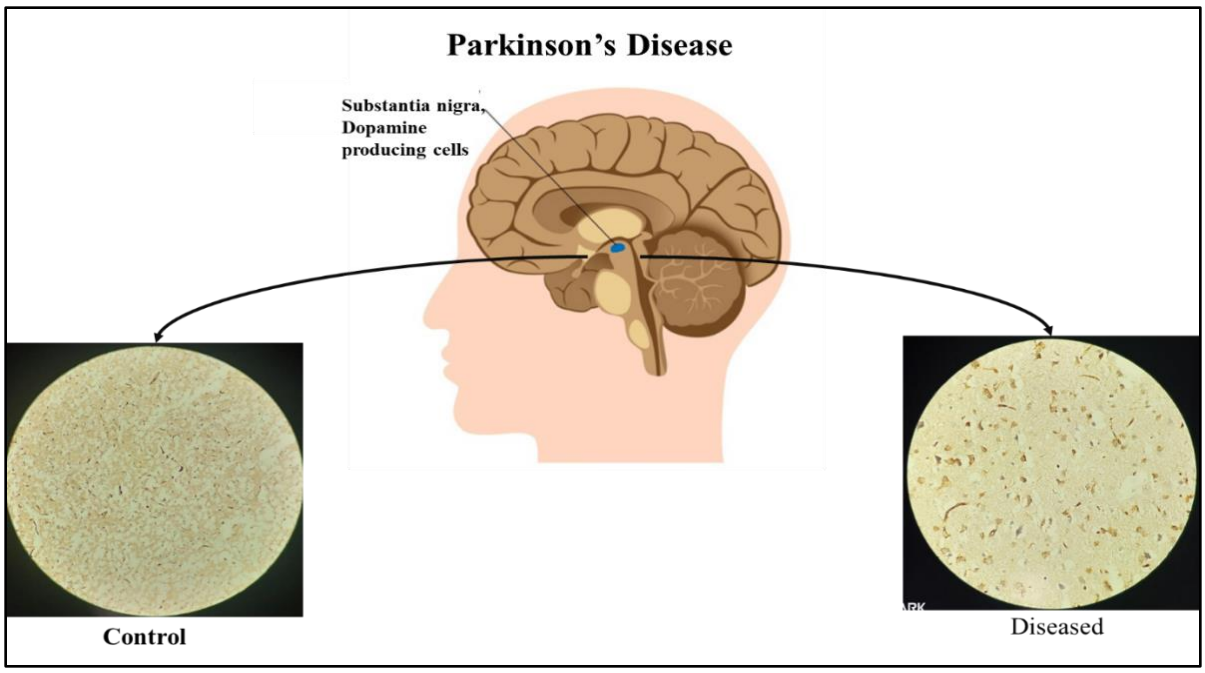

Figure 4.9: Visualization of SNpc GFAP Staining

Substantia nigra also contains astrocytes which are GFAP positive. In diseased group, after MPTP administration, increased level of GFAP positive astrocytes were observed in striatum and substantia nigra. GFAP- Moderate (++) positivity in striatum and substantia nigra. In contrast, control group showed GFAP-Mild positivity was observed.

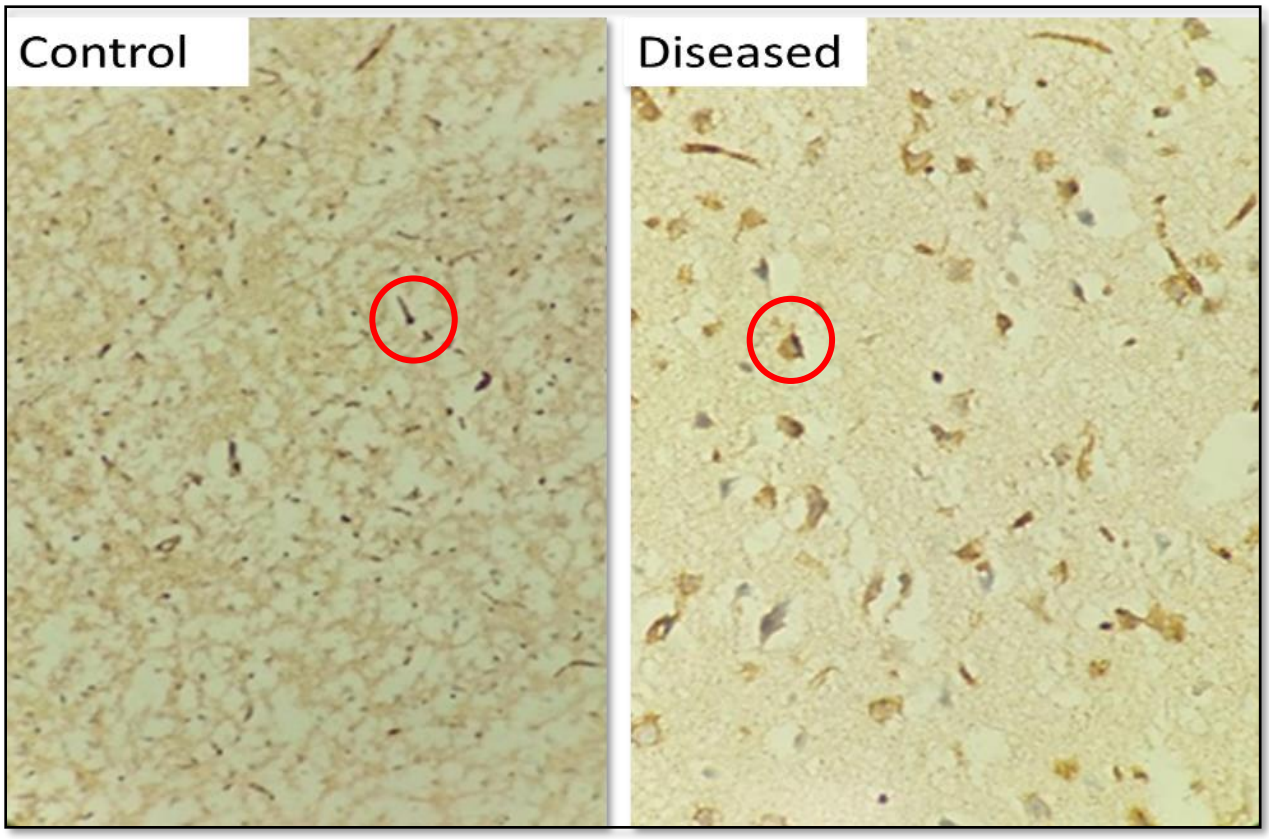

Figure 4.10: Visualization of SNpc GFAP Staining In diseased group, after MPTP administration, increased level of GFAP positive astrocytes were observed in striatum and substantia nigra. GFAP- Moderate (++) positivity in striatum and substantia nigra. In contrast, control group showed GFAP-Mild positivity was observed.

CHAPTER FIVE: DISCUSSION

The primary pathology underlying Parkinson's disease (PD) is the loss of dopaminergic neurons in the substantia nigra (SN). Oxidative stress is a component of the MPTP-induced neurotoxicity mechanism. Increased oxidative stress and pro-inflammatory reactions have a significant role in causing or accelerating nigrostriatal degeneration. The dopaminergic system most commonly impacted by PD is the nigrostriatal pathway. The cell bodies and nerve terminals of the neurons that make up this circuit are located in the SNpc and striatum, respectively. A variety of phenotypic changes that astrocytes and microglial cells go through after a brain injury allow them to react to and participate in the pathological processes.

GFAP, also known as glial fibrillary acidic protein, is an intermediate filament protein that emerged at initial stage in the progression along with the emergence of several glial cell types in the central nervous systems. The discovery of novel intermediate filament activities and significant recent advancements in astrocyte biology sparked interest in GFAP's role in biology. The finding of multiple GFAP splice variants provided another impetus to investigate this protein in greater depth. For appropriate astrocyte function, the cytoskeleton's stability is crucial.

GFAP levels in substantia nigra increases when there has been an injury or disease, often to startling levels. This is especially true in cases of traumatic brain injury, stroke, subarachnoid hemorrhage, and neuromyelitis Optica. Neuro-degenerative diseases, where GFAP is the disease initiator and is likewise high, may benefit especially from observation of cells from substantia nigra and striatum

Neural damage correlate with increased GFAP immunoreactivity. The result of this study indicates that diseased group, after MPTP administration, increased level of GFAP positive astrocytes were observed in striatum and substantia nigra. GFAP- Moderate (++) positivity in striatum and substantia nigra. In contrast, control group showed GFAP-Mild positivity was observed.

H & E staining of control group showed normal nucleus as well as cytoplasmic area in normal pattern, shape and structure of cells. No neuron loss is noted. In contrast, H & E staining of diseased

group showed altered morphology of nucleus and cytoplasmic area than normal which demonstrates the disease. It showed cluster of foamy histiocytes. Loss of neurons was observed in this region.

In neurological diseases with inflammation. GFAP is a promising marker to identify persons at risk and enable swift implementation of future preventive, and eventually curative strategies in the older population because it appears to predict the rate of cognitive deterioration and conversion to overt dementia. Last but not the least, new information suggests that GFAP may be able to monitor even minute structural CNS involvement in a variety of neurological and systemic illnesses.

Academic partnerships might considerably speed up efforts to close knowledge gaps and ease the widespread use of GFAP as a biomarker. Future research should focus on developing a new therapeutic strategy to reduce the level of GFAP in the substantia nigra and striatum in Parkinson's disease. This will be done by employing the GFAP pathway and a targeted medication or protein.

CHAPTER SIX: CONCLUSION

MPTP has been found as a neurotoxin that induces irreversible Parkinson's disease symptoms by damaging dopaminergic neurons specifically in the substantia nigra region. Disease model is established in mice. Some major modifications were demonstrated in the behavioural activities and motor functions of MPTP-induced PD mouse model. A modified stimuli causes Inflammatory responses that is demonstrated by the upregulation of the GFAP. Homeostasis as well as the water and ion maintenance are considered as the significant functions of astrocytes. During disease phase, a significant increase in GFAP that is considered as an astrocyte-specific marker was observed. In our model, Upregulation of GFAP as well as cell body’s hypertrophy with its extensions has been observed which is a characteristic of reactive astrogliosis. Also, in some affected brain areas of PD patients, reactive astrogliosis are identified that stipulating the collaboration of astrocytes in immunity.

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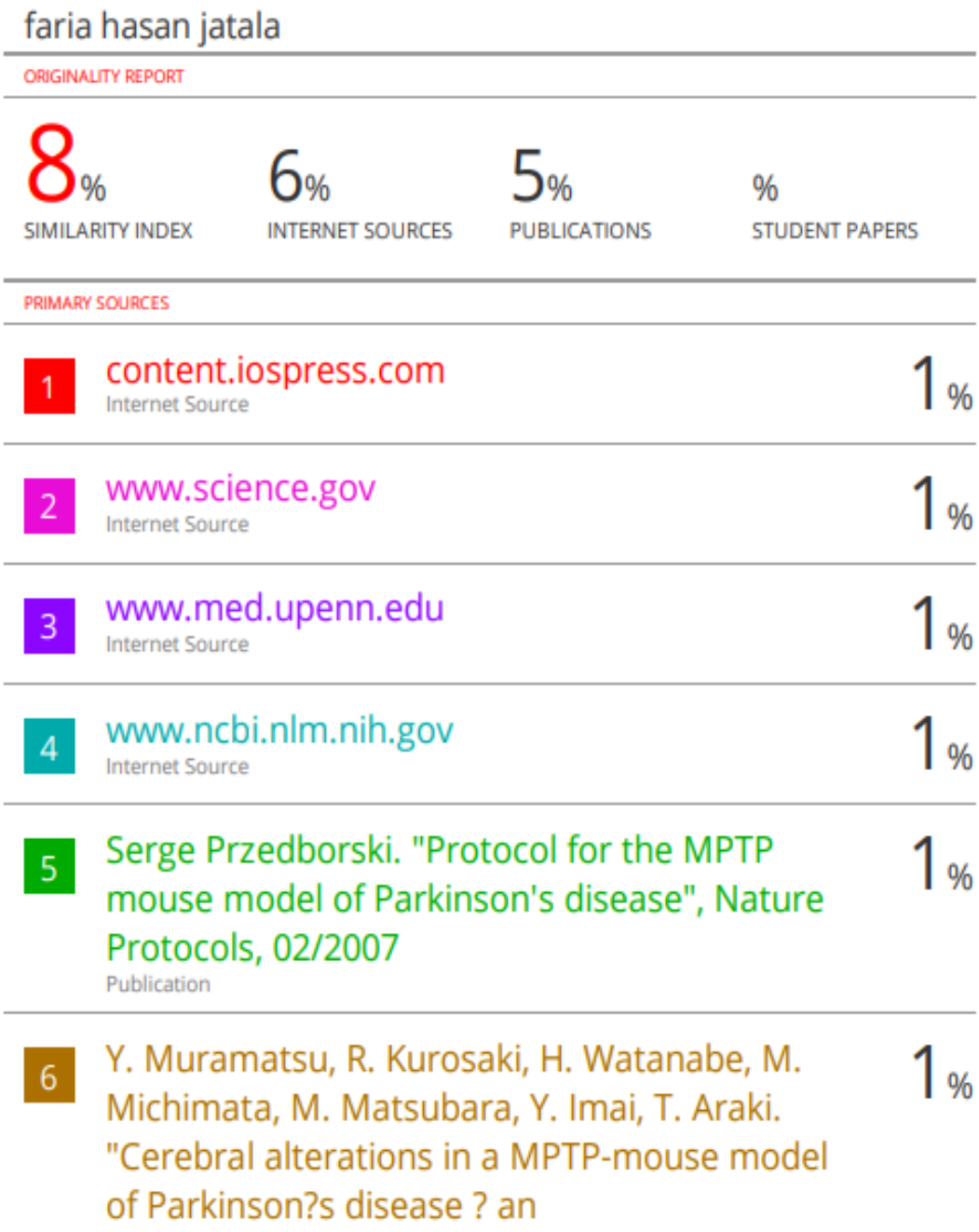

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