Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon/ College of Medicine

Evaluation the antiparkinsonian Effect of Salvia officinalis on rat animal model of Parkinson's disease

A Thesis Submitted to the College of Medicine / University of Babylon as Partial Fulfillment of the Requirements for the Degree of Master in Pharmacology / Pharmacology and Toxicology

By:

Zainab Oleiwi Sabeeh

BSc. Pharmacy (2014-2015)

Supervised by:

Assistant Professor

Dr. Selman Mohammed Selman

M.B.Ch.B., PhD (Pharmacology)

Assistant professor

Dr. Asmaa Mohammad Makkey

PhD (Histology)

1443 A.H **2022 A.D**

بسم الله الرحمن الرحيم

صدق الله العظيم

سورة المجادلة آية 11

Supervision Certificate

We certify that this thesis entitled "Evaluation the antiparkinsonian effect of *Salvia officinalis* on animal model of Parkinson's disease " was prepared under our supervision in the department of pharmacology as a partial fulfillment for the master degree of Pharmacology and Toxicology.

Assistant Professor

Dr. Selman Mohammed Selman

Supervisor

Assistant Professor

Dr. Asmaa Mohammad Makkey

Supervisor

In view of the available recommendation, we forward this thesis for debating by examining committee.

Assistant Professor

Dr. Selman Mohammed Selman

Chairman of the Department of Pharmacology

College of Medicine / University of Babylon

ACKNOWLEDGEMENTS

All praise be to Almighty Allah, Lord of the Universe, and next to His Prophet, Mohammed, his daughter Fatima al-Zahra', and the Twelve Infallible Imams, prayers and peace be upon them all.

I would like to express my sincere gratitude to my supervisors assist. Prof. Dr. Selman Mohammed Selman, and assist. Prof. Dr. Asmaa Mohammad Makkey for their consistent support and guidance during the research.

Special thanks for Dr. Dalia alshereify for helping in my work.

With my great thanks to my family specially my dear mother and father for support.

And my big appreciation for my dear husband who his encouragement helps me alot and for his patience, assistance during my study.

Summary

parkinson’s disease (PD) is an intractable disease resulting in localized neurodegeneration of dopaminergic neurons of the substantia nigra pars compact (SNc) Usually, both selective degeneration of dopaminergic neurons in the *substantia nigra* pars compacta (SNpc) and the appearance of Lewy bodies, whose main component is aggregated α-synuclein, in the remaining neurons are thought to be the major pathological hallmarks of PD.

This study was aimed to evaluate the effects of salvia officinalis extract on the brain of animal model of Parkinson disease, and if it can show better results when given synergistically with other parkinson’s treatments due to knowledge provided from previous studies about anti oxidant and anti-inflammatory properties of salvia officinalis.

Fifty male rat where divided in to five groups, group 1 (control) does not exposed to rotenone nor receive any treatment only given normal saline for 30 days, group 2 was injected by rotenone (2.5 mg/kg) IP for 30 days without any treatment, group 3 where injected with rotenone(2.5 mg/kg) and received sinemet tablet by gavage after dissolving it with water on day 15 for 30 days also, group 4 where injected with rotenone(2.5 mg/kg) and received extract of S. Officinalis (500 mg/kg)orally by gavage on day 15 for 30 days, group 5 where injected with rotenone(2.5 mg/kg) and received extract of S. Officinalis (500 mg/kg)and sinemet tablet orally by gavage on day 15 for 30 days, the antiparkinson effect of salvia officinalis and sinemet where evaluated by using open field test, force gripping test, and rotarod test.

At the end of the experiments, animals were sacrificed by decapitation, the malondialdehyde(MDA) was determined by enzyme linked immunosorbent assays kits (Elisa) in rats blood. and number of neurons cells preserved with tyrosine hyrdroxylase(TH) enzyme and it’s intensity were determined by immunohistochemical studies on rats brain tissues.

In group 2, 4, 5 there was significant decrease in weight comparing with group 1 and in group 2,3,4 there was significant decrease in no. Of rotations,

rotation distance, time of rotation comparing with group1. While in group3,4,5 there was significant increase as compared with group2 in term of rotarod test (P-value <0.05).

In group 2, 3, 4 there was significant decrease in crossing, rearing, grooming time, no. Of visits to central area comparing with group1. While in group3,4,5 there was significant increase as compared with group2. in term of open field test (P-value <0.05).

Furthermore,In group 2, 3, 4 there was significant decrease in force gripping as compared with group1 . While in group3,4,5 there was significant increase as compared with group2 In force gripping test (P-value <0.05).

In biochemical test there was significant increase in MDA level in group 2 as compared with group 1. And significant decrease in it's level in group 3, 4, 5 as compared with group 2 (P-value <0.05).

In immunohistochemical studies the no. Of dopaminergic neurons with +ve tyrosine hydroxylase enzyme was significantly decreased in group 2, 3, 4, 5 as compared with group 1. While there was significant increase in group 3, 4, 5 as compared with group 2,(P-value <0.05). And the histoscore of intensity of TH in neurons preserved in dopaminergic neurons was significantly decreased in group 2, 3, 4, 5 as compared with group 1. While there was significant increase in group 3, 4, 5 as compared with group 2.

List of contents

List of Tables

List of Figures

List of Figures

List of Pictures

List of Abbreviations

Abbreviations	Meaning
6-OHDA	6-hydroxydopamine
AD	Alzheimer's disease
AADC	aromatic amino acid decarboxylase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood brain barrier
CNS	central nervous system
COMT	Catechol-Omethyl transferase
CCl4	Tetrahydrochloride
D.W.	Distilled water
DA	dopamine
DA-ergic	Dopaminergic
DOPAC	3,4-dihydroxyphenylacetic acid
DMT1	Divalent metal transporter 1
D1R	Dopamine receptor type 1
D5R	Dopamine receptor type 5
DMSO	Dimethyl sulfoxide solution
DAB	3,3′-Diaminobenzidine
DOPAC	3,4-dihydroxyphenylacetic acid
DJ-1	Protein/nucleic acid deglycase
Elisa	enzyme linked immunosorbent assays
FDA	Food and Drug Administration
Fe2+	Ferrous iron
Fe3+	Ferric iron
GSH	Glutathione
GABA	Gamma aminobutyric acid
g	Gram
H2O2	Hydrogen peroxide
H+	Proton
HD	Huntington's disease
HVA	homovanillic acid
HIC	Hydrophobic interaction chromatography
IL-1	Interleukin-1

IL-8	Interleukin-8
IL-33	Interleukin-33
IHC	Immunohistochemical study
IP	Intraperitoneal
LOOH	plasma lipid hydroperoxides
LBs	Lewy bodies
LPS	Lipopolysaccharide
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2
MPTP	1-methyl-4-phenyl tetrahydropyridine
MDA	malondialdehyde
Mg	Milligram
MAO	Monoamine oxidase
MAO- B	Monoamine oxidase B
MAOIs	Monoamine oxidase inhibitors
NMS	non-motor symptoms
NADPH	Nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor kappa beta
NMDA	N-Methyl-D-aspartic acid
O2.	Molecular oxygen
O2·-	Superoxide radical
OH−	Hydroxide ion
PBS	Phosphate buffer solution
PD	Parkinson disease
PINK1	Serine/threonine-protein kinase
PQ	Paraquat
rpm	Round per minute
RNS	reactive nitrogen species
ROS	Reactive oxygen species
SOD	superoxide dismutase
S.O.	Salvia officinalis
SEM	Standard error of the mean
SN	substantia nigra
SNpc	substantia nigra pars compacta
SNCA	α-synuclein
SPSS, v20	Statistical Package of Social Sciences, version 20
TH	Tyrosine hydroxylase
TNF	Tumor necrotic factor

Chapter One

Introduction

Parkinson's disease (PD) is an incurable condition that causes the dopaminergic neurons of the substantia nigra pars compact to degenerate locally (SNpc) (Theo Stoddard-Bennett 2018). Typically, Lewy bodies, whose main component is aggregated - α-synuclein, emerge in the surviving neurons and selective degeneration of dopaminergic neurons in the (SNpc) are regarded to be the principal pathological characteristics of PD (Przedborski, 2017; Johnson et al., 2019). Parkinson's disease is a recognisable clinical syndrome with a range of causes and clinical presentations. Parkinson's disease represents a fast-growing neurodegenerative condition; the rising prevalence worldwide resembles the many characteristics typically observed during a pandemic, except for an infectious cause.(Dorsey ER , Sherer T2018) There are an estimated 6 million people affected by PD worldwide, making it the second most prevalent neurodegenerative condition. (Darweesh SK, Koudstaal PJ, JAMA Neurol 2016). Between 1990 and 2016, there was a 74% increase in the prevalence of PD, and until 2030, there will likely be another 2- to 3-fold increase. Both motor and non-motor function gradually deteriorates as a result of the disease. The three fundamental motor characteristics of bradykinesia, rigidity, and tremor of the distal extremities make up the motor symptoms of Parkinson disease. (.Jankovic J, Goodman I 2018) The core symptom of Parkinson's disease (PD), bradykinesia, is characterized by decreased movement speed and amplitude, with the latter often deteriorating with repetitive motor patterns.The risk of developing PD is twice as high in men than in women; particularly, women have a higher mortality rate and

faster progression of the disease (Cerri, S.; Mus, L.; B2019). The massive loss of dopaminergic neurons that extend from the SN to the striatum is the primary source of these symptoms. (Dhanalakshmi, et al. 2016)and(Duce, et al. 2017). Conventional medicines frequently fail to treat advanced PD, which is made more difficult by both motor and non-motor issues. The importance of non-motor symptoms (NMS) has increased in recent years due to their significant impact on quality of life indicators and societal expenses associated with PD. Changes in mood, cognitive deterioration, pain, disturbed sleep, and autonomic dysfunction are among these symptoms. (Chaudhuri KR, Schapira AH2009) However, several of these symptoms, such as sensory issues, depression, constipation, and sleep disturbances, can appear early in the course of the disease, even at a premotor stage. Non-motor symptoms have also been observed to correlate with advancing age and severity of PD. (Chaudhuri KR, Yates L, 2005) As a quantitative measure of prevalence, the expanding variety of motor and non-motor symptoms associated with PD has been described, demonstrating the clinical breadth of the illness. (Schapira AHV, Chaudhuri KR,2017)

1. 1. 1. Aims of the Study

This study was aimed to:

1. Finding out whether S. officinalis plays a role in attenuating the PD symptoms in male rats.

2. Identifying whether S. officinalis is more effective alone or with sinemet tablet in attenuating the PD signs in male rats.

3. Figuring out the role S. officinalis plays in PD.

4. Identifying the effect of S. officinalis on the levels of tyrosine hydroxylase and MDA.

5. Determining whether S. officinalis is able to protect the brain tissue in male rats against development of PD.

1. 1.2.Hypotheses

This study hypothesizes that:

1. S. officinalis plays an important role in attenuating the PD symptoms in rats.

2. S. officinalis plays an important role as an anti-oxidant and anti-inflammatory agent in PD.

3. S. officinalis is effective in increasing the levels of tyrosine hydroxylase and decreasing the level of MDA.

1. 2. Literature review

Parkinson disease (PD) is the second most prevalent, progressive age-related neurodegenerative disease. It usually strikes people in their late 50s and early 60s, while uncommon variants of the disease can strike people as young as 40. The prevalence of the disease increases with that of age making it the major risk factor of PD (Peshattiwara, et al. 2020). Parkinson disease is defined as a neurodegenerative disorder characterized by gradually occurring dopaminergic neuronal cell death in the substantia nigra par compacta(SNpc). The four cardinal symptoms are tremors, bradykinesia(slowness of movement), rigidity (muscle inflexibility)and postural instability(Balance impairment affecting a person's ability to change or maintain postures). The name PD is acquired after James Parkinson, who reported many of the clinical features of the disease in his works entitled "Essay on the shaking palsy "in 1817. The major symptoms occur due to brain nigrostriatal system degeneration. Apart from the depletion of dopaminergic neurons in the brain's SNpc, it is also followed by reducing in striatal dopamine (DA) levels and its metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)(Hussain and Aliuddin 2018). Additive non-motor symptoms include cognitive deficits, sleep disturbances, anxiety, motivation disorders and mood disorders (Duce, et al. 2017).The existence of SNCA-containing Lewys bodies (LBs)in the SN of the brain is a neuropathological marker for PD. The lack of DA-ergic neurons in the SNps causes a reduction in voluntary movement facilitation. SNCA accumulation spreads across the brain as PD progresses. Over the last 10–20 years, non-motor symptoms in PD have been given considerable attention. Nevertheless, the motor signs of the disease making PD a movement

disorder, still represent the hallmarks of the disease and are the most important characteristics for a diagnosis of PD, even today with modern imaging or laboratory tests to help with the diagnostic challenge (Storstein,et al. 2017).

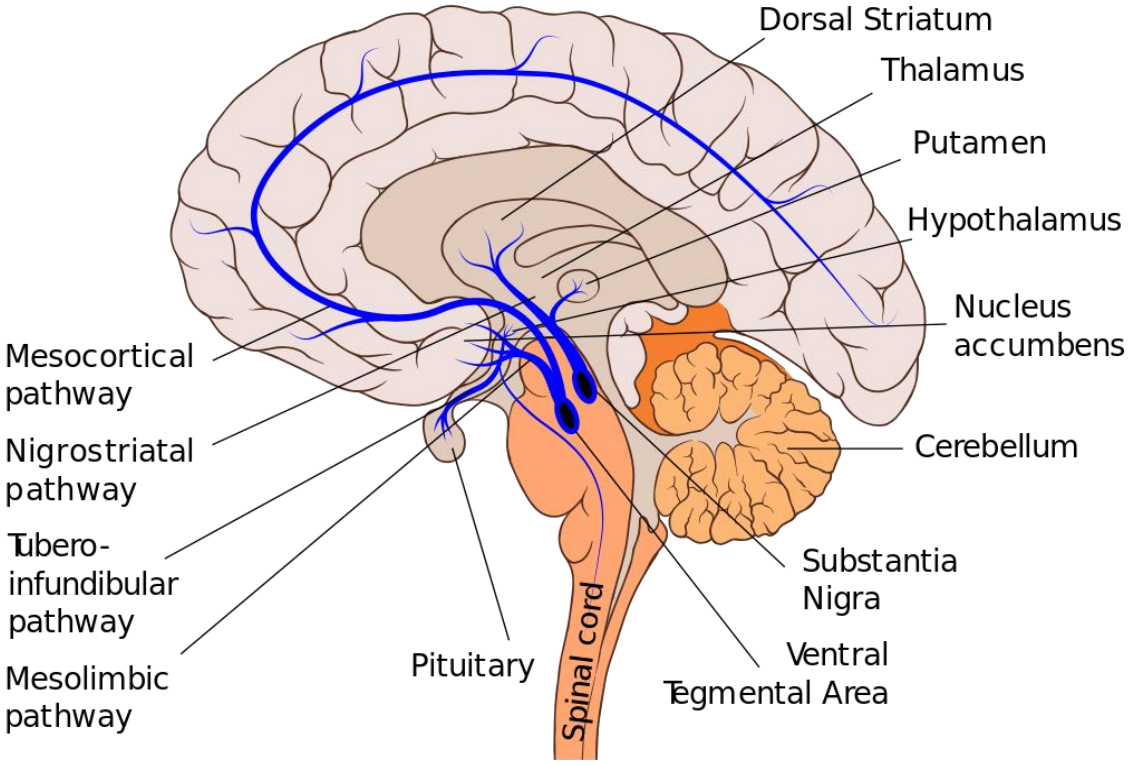


Figure 1.1: dopaminergic pathways in the central nervous system. (*Alcaro A, Huber R, Panksepp J (December 2007)).*

1.3. Etiology of Parkinson disease

Researchers have been mentioned the etiology of PD. Direct comparison of prevalence estimates is challenging due to methodological discrepancies across studies. It is generally accepted that the prevalence of the disease ranged from 1 to 2 per 1000 in unselected populations and that the disease affects 1%of the population above 60 years(Connolly and Lang 2014). PD is rare before the age of 50, and it has a frequency of 4% in the oldest age categories. The yearly incidence per 100,000 people varies between fewer

than ten and more than twenty. PD occurs sporadically and the true etiology is unknown (Storstein, et al. 2017).The incidence appears to be greater in males than in female (the ratio ranges from 1.3 to 2.0), but the incidence may be influenced by differences in prevalence of variables such as smoking of cigarette, using of postmenopausal hormone and caffeine (Joseph and Tan 2020).

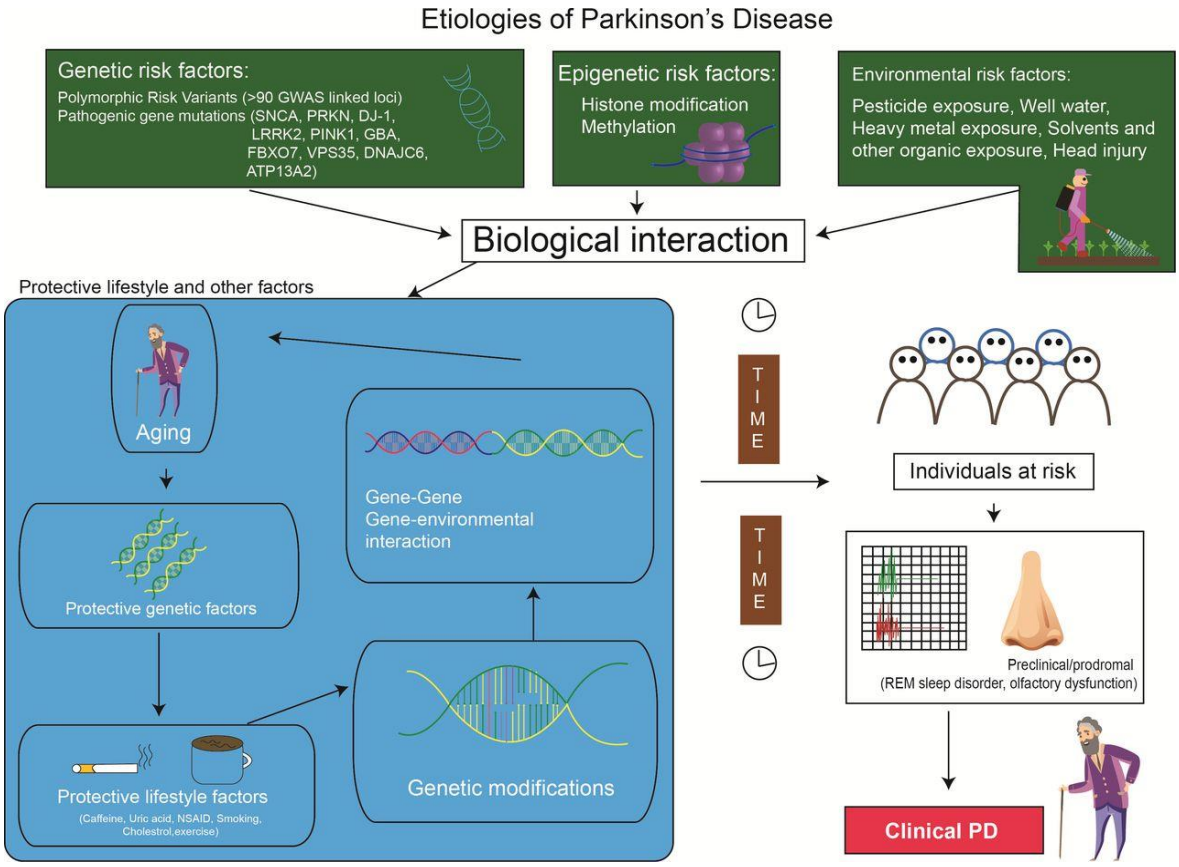


Figure 1.2: Etiologies of Parkinson disease. (Joseph and Tan 2020).

1.4.Epidemiology

Parkinson disease is thought to affect 0.3 % of people overall in developed nations, 1.0 % of individuals over 60, and 3.0 percent of people over 80; incidence rates are thought to range between 8 and 18 per 100,000 people-years. (Lee A, Gilbert RM. 2016). While the estimated prevalence and incidence rates for Parkinson's disease (PD) in Europe range from 65 to 12,500 per 100,000 and 5 to 346 per 100,000 person-years, respectively. (von Campenhausen S, Bornschein B,2005). Age is the main risk factor for the condition, and men are at a moderately higher risk than women. (Gillies GE, Pienaar IS,2014). Several environmental factors, such as particular pesticides and living in a rural area, have been related to the risk of PD. (Breckenridge CB, Berry C, 2016). It's notable that some agents, including 1-methyl-4-phenyl tetrahydropyridine (MPTP) and annonacin, can lead to nigrostriatal cell death.

1.5. Pathogenesis of Parkinson disease

There are Environmental and hereditary factors are thought to involve in the pathogenesis of PD. The degenerated dopaminergic neurons (axons and soma) that propagating from the SNc to the striatum is a characteristic of PD at the cellular level (Duce, et al. 2017). Neurons in the autonomic ganglia, the enteric nervous system, the limbic system, the olfactory bulb, the spinal cord, and the neocortex are also impacted. The underlying mechanisms are complex and interconnected, involving toxic biochemical reactions (excitotoxicity, nitric oxide toxicity, oxidative stress), abnormal cellular and cell death signaling pathways (apoptosis, inflammation), dysfunctional organelles (lysosomes, mitochondria), and dysfunctional protein degradation systems (autophagy, ubiquitin proteasomal

system), all of which led to cell death. Several of these systems result in an excessive release of free radicals, which cause cell stress by destroying membranes and organelles (DiPro, et al. 2020).

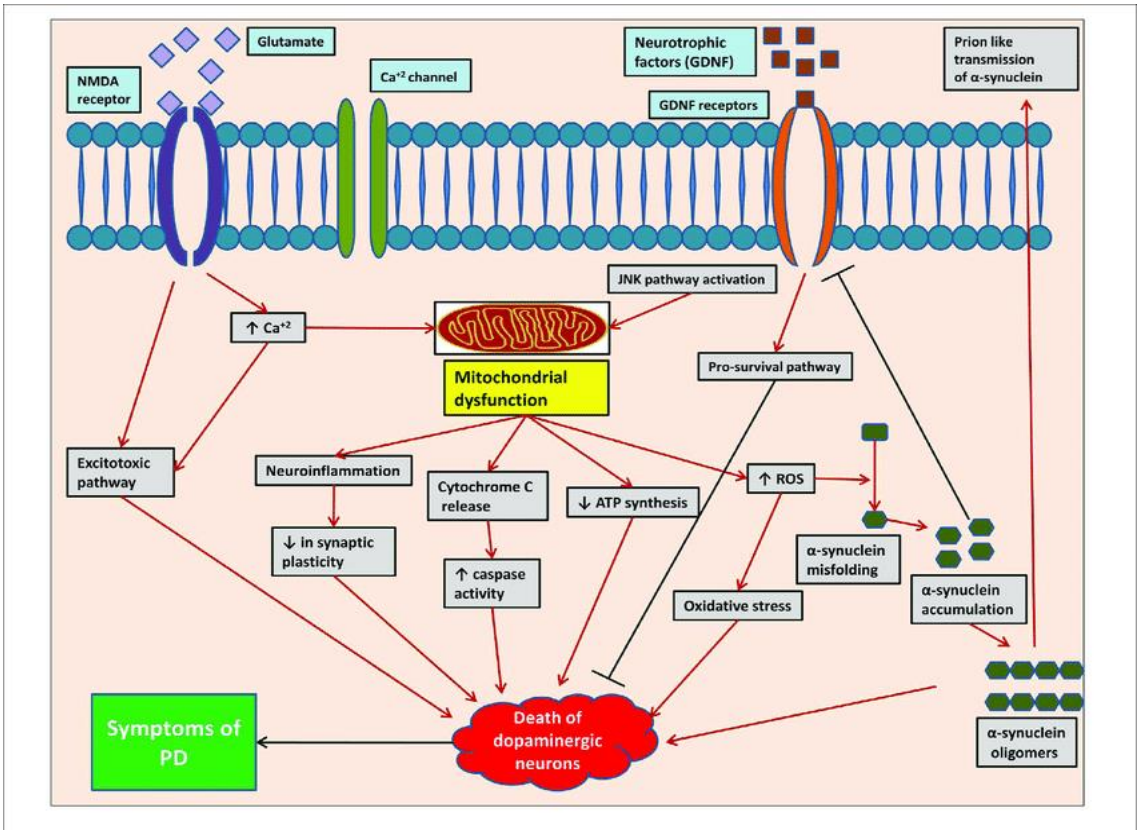


Figure1.3: A scheme illustrating Pathogenesis of Parkinson's disease at molecular level, showing different complex mechanistic singling pathway/events.(Hammad and Haroon 2018)

1.6. Pathophysiologic mechanisms

The loss of dopaminergic neurons in the SN is a characteristic of Parkinson disease. The Lewy body, a neuronal inclusion primarily made up of α -synuclein protein aggregations, is the pathologic feature of Parkinson's disease. The Braak hypothesis is the most frequently referenced theory to explain the neuropathological development of Parkinson disease. (Braak H, Del Tredici K2003) According to this hypothesis, the medulla and the olfactory bulb which is where Parkinson disease (stages 1 and 2) first manifests itself. This early pathology is linked to symptoms including diminished smell and rapid eye movement sleep behavior disorder, which causes people to lose their typical rapid eye movement sleep paralysis and physically behave out their dreams while they are asleep. The substantia nigra pars compacta, along with other midbrain and basal forebrain regions, are affected in stage 3 and stage 4 of the disease. Motor symptoms of Parkinson disease are correlated with pathology in these regions. At this point, Parkinson disease is often identified. When Parkinson disease is progressed, cognitive decline and hallucinations start to appear when the pathology spreads to the cerebral cortices. (Braak H, Del Tredici K2003). in late stages, the cortical regions eventually become affected (Braak stages 5 and 6). These include SNCA misfolding and aggregation, mitochondrial dysfunction, impairment of protein clearance (involving key ubiquitin-proteasome and autophagy-lysosomal systems), neuro-inflammation and oxidative stress (Joseph and Tan 2020) The several cellular pathways that contribute to PD-related neurodegeneration in the context of oxidative stress, age, lifestyle/environmental, and genetic variables.

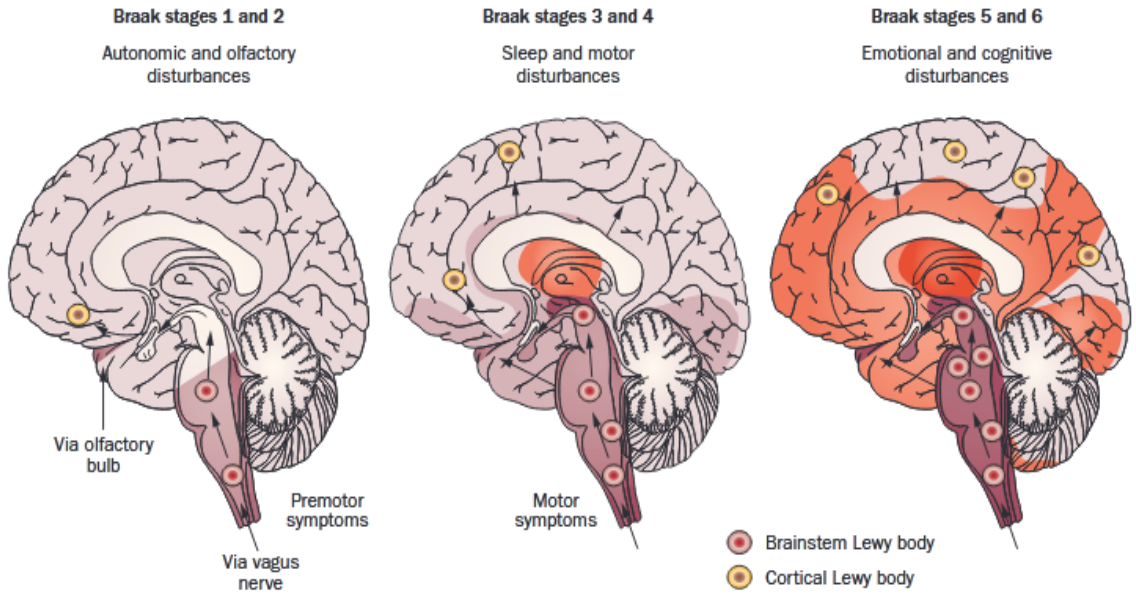


Figure 1.4: Braak staging. (Richard L. Doty2012).

1.6.1. Oxidative Stress

Oxidative stress plays an crucial role in the degeneration of dopaminergic neurons in Parkinson disease leading to disruption of physiologic maintenance of the redox potential in neurons by interfering with several biological processes, ultimately leading to cell death (Dias V, et al. 2013).There are two basic processes that cause oxidative stress. The first is the ROS pathway enzymes tyrosine hydroxylase and monoamine oxidase, which are responsible of making dopaminergic neurons exposed to oxidative injury. The presence of iron causes the fenton reaction, which increases oxidative stress by generating super oxide radicals and hydrogen peroxide, in nigral dopaminergic neurons, which is the second mechanism (H2O2) (Khan and Ali 2018). Reactive oxygen species, depending on the subcellular location of ROS generation, can cause oxidative damage to lipids, proteins, DNA, and

RNA, impairing neuronal function and structural integrity. This suggests that excessive ROS formation may be a primary cause of the death of dopaminergic neurons rather than a subsequent reaction to advancing neurodegeneration. (Benjamin, Dominic and Kay 2019).The SNc and the striatum are regions characterized by increasing levels of oxidative stress as a result to DA degradation and the Fenton reaction. In the Fenton reaction, H2O2 accepts an electron from ferrous iron (Fe2+) to produce ferric iron (Fe3+) and the hydroxyl radical (HO*). Fe3 is reduced back to Fe2+by another molecule of H2O2, forming a hydroperoxyl radical (HOO*). The radicals damage cell membranes and organelles (eg.mitochondria) and also induce apoptotic signaling (DiPro, et al. 2020).

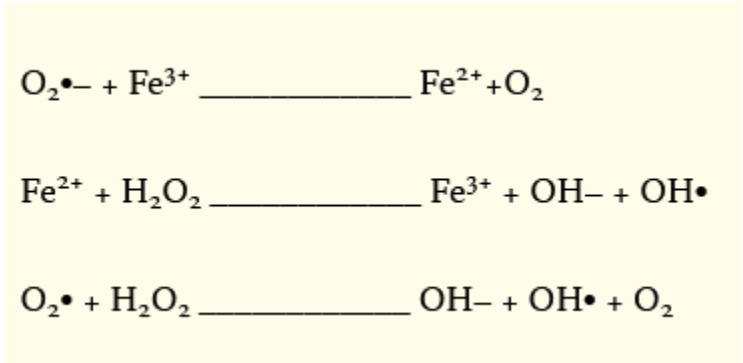


Figure 1.5: fenton reaction (Genaro Gabriel, Fermín P.2016)

Oxidative stress is promoted preferentially in the SNc by changes in DA, iron, and SNCA. SNCA aids "dynamin-mediated endocytosis of iron bound holo-transferrin and transferrin receptor" in physiological conditions. A facile cytoplasmic labile iron pool should be closely controlled by ferritin to make ferroprotein do its function, such as dopamine production by tyrosine hydroxylase (TH). SNCA promotes several processes in the release, reuptake, and packing of dopamine into synaptic vesicles at synapses. Divalent metal transporter 1 (DMT1), which is not controlled by intracellular iron levels,

must be used because oxidation and phosphorylation of α -synuclein restrict transferrin receptor-mediated iron entry in Parkinson's disease (PD). This, along with a decline in "ferritin's iron storage capacity" due to aging, raises the labile iron pool, which take part in "Fenton chemistry" and produces ROS when it combines with free DA (Duce, et al. 2017). Impaired DA packaging into synaptic vesicles and decreased synaptic DA release, both of which are connected to abnormal SNCA posttranslational modification, result in increased free DA levels.Lewy pathology deposition is associated with SNCA oxidation and phosphorylation, which exacerbates oxidative stress in the nigral region. (Benjamin, Dominic and Kay 2019).There are two strategies to measure oxidative stress. The first strategy is to use oxidative stress biomarkers, and the second is to calculate antioxidant levels in a cell. Reduced activity of glutathione, catalase, and superoxide dismutase (SOD), and other antioxidant enzymes, as well as higher amounts of free radicals, protein and lipid oxidation, and DNA damage (GSH), make DAergic neurons in PD patients more vulnerable to oxidative stress. Patients with PD undergo both elevated and reductioned activity in their SNpc neurons, which causes oxidative stress and, in turn, neuro-inflammation. (Khan and Ali 2018).

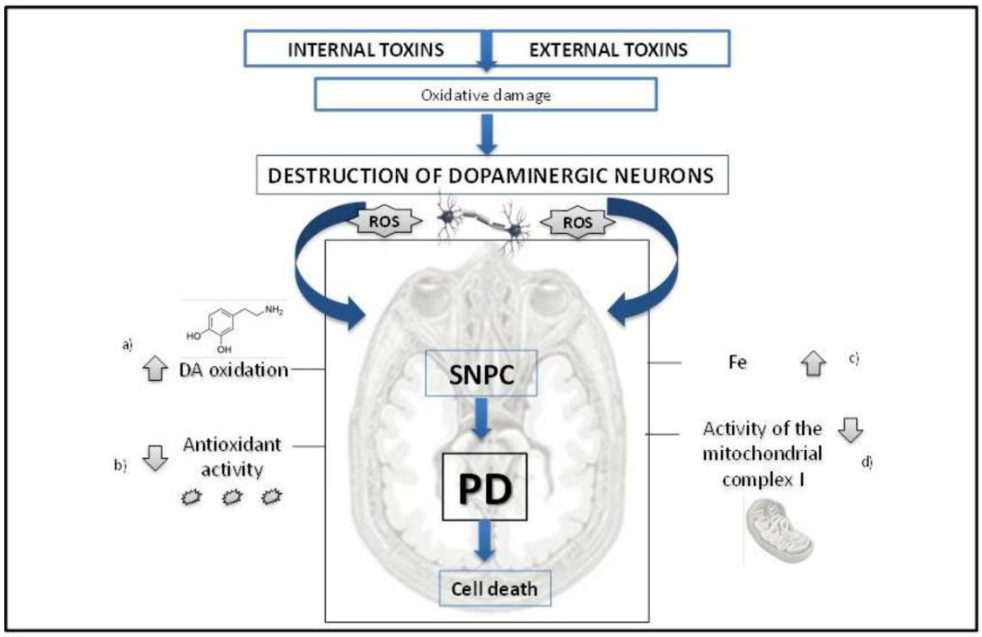


Figure 1.6: SNPC neurons and oxidative agents.

Different pathways contribute to the substantia nigra pars compacta (SNPC) neurons vulnerability to oxidative damage including (a) high susceptibility of DA auto-oxidation, (b) reduced antioxidant activity such as glutathione, and (c) increased iron concentration d) deficits in mitochondrial complex I of the respiratory chain. (Genaro Gabriel, Fermín P.2016).

1.6.2. Striatal Pathophysiology of Parkinson disease

The nuclei in the basal ganglia play a significant role in the determination of motor and cognitive actions. Dopamine from the striatonigral pathway regulates the cortex and the thalamus, which are synaptically connected to striatal neurons and spiny projection neurons.. Inhibitory spiny projection neurons, which innervate various target nuclei, make up about 90% of striatal cells. These neurons have either D1 or D2 DA receptors. (Calabresi,et al. 2014). Reducing the amount of the primary neurotransmitter DA in the striatonigral pathway apparently causes an

imbalance in the striatal output, which then compromises the cortex's ability to fire normally through D1 spiny neurons in the thalamus, which effect the normal movement of the arms that innervated by D1 spiny neurons. Therefore, rather than only being caused by a drop in dopamine, it is possible that the cognitive and motor difficulties frequently observed in PD patients are caused by the breakdown of information transmission via the striatonigral synaptic pathway. (Bolam and Pissadaki 2012).The reason that SN is the target of the high degree of oxidative stress in PD may lie in its high energy metabolism and the high content of DA in its neuronal cells, although DAergic cells are normally endowed with a huge range of protective mechanisms, disrupting the equilibrium between free radical formation and neutralization by a complex antioxidant system(Sofic, et al. 2006).

1.6.3. Lipid Peroxidation and Parkinson disease

Lipid peroxidation damage membrane structure and impairs protein and DNA function. many studies demonstrate altered levels of products of lipid peroxidation, such as isoprostanes and malondialdehyde (MDA), in brain tissues of neurodegenerative patients. MDA levels have been found to be high in the plasma of PD patients (Chang and Chen 2020). Increased basal lipid peroxidation may result in inhibition of DA synthesis and damage to the DA synthetic systems. A significant increase of MDA formation, expressing the rate of lipid peroxidation, has been shown in the SN of PD brain (Sofic, et al. 2006).MDA is the best biomarker for PD, although LOOH (plasma lipid hydroperoxides) and SOD activity are related with late-stage PD but not early-stage PD. So new treatments for PD should target not only DA but also lipid peroxidation (Fariasa,etal. 2016).

1.6.4.Neuroinflammation in Parkinson's disease

Neuroinflammation plays major role in several neuro-degenerative diseases, including Parkinson's disease (PD), Huntington's disease (HD), and Alzheimer's disease (AD) (Aid and Bosetti2011). In the central nervous system (CNS), an acute neuroinflammatory response is desireable and promotes the repair of damaged tissues; however, a persistent neuroinflammatory response contributes to brain damage. (Eikelenboom et al.2010). Generally, neuroinflammation is strongly linked to neuronal damage and cell death via a variety of biological mechanisms, including increased oxidative stress and activated glial (astrocyte and microglia) cells. (Agostinho et al.2010; Niranjan2014). In case of neuroinflammation, activated glial cells discharge pro-inflammatory and neurotoxic substances that cause neuronal degradation and impairment. (Harry andKraft2008). As a result, the neuroinflammatory process is linked to reactive gliosis activation and neuronal death as well as immune cell infiltration and microglia as primary immune cells. These cells produce and release pro-inflammatory substances such cytokines, prostaglandins, complement proteins, reactive nitrogen species (RNS),and reactive oxygen species (ROS), which can increase brain damage when it occurs in pathological settings. (Agostinho et al.2010). In addition, glial activations during the pathophysiology and development of PD may help treat damaged tissues and control inflammation.

1.6.4.1.Mediators during Neuroinflammation

cytokines, chemokines, and Inflammasome, play a critical role in triggering neuroinflammation, and as potential therapeutic targets, .Cytosolic multi-protein complexes are called inflammasomes. that act as intracellular

sensors for both microbial pathogens and foreign as well as host-derived danger signals, activating the pro-inflammatory caspase-1 (a family of cysteine-dependent aspartate specific proteases that play a central role in inflammation and programmed cell death) It's function is controlling the maturation and release, of the inflammatory cytokines IL-1, IL-18, and IL-33. as well as stimulation of the pyroptotic process (Voet, et al. 2019).The pro-inflammatory cytokines have been shown to stimulate a wide range of innate immune processes associated with infection, inflammation, and autoimmunity, and so act as a key role in inducing neuroinflammation in old age, as well as the development of cognitive defects, neurodegenerative disorders, and dementia (Gaurav Singhal, et al. 2014). IL-1β, IL-6, and TNF-α one of the most extensively studied pro-inflammatory cytokines, IL-4 and IL-10 are well-known anti-inflammatory cytokines (Cordaro, Cuzzocrea and Crupi 2020).The IL-1 family of cytokines comprises 11 secreted factors, including IL-1α, IL-1β, IL-18, and IL-33, which are known for playing a role in host defense and immune system regulation in inflammatory diseases. These cytokines have been implicated in a wide range of immunological responses, as well as in the initiation, regulation, and maintenance of inflammation. In spite of that pro-inflammatory cytokines have been demonstrated to cause neurodegenerative disorders at high levels of expression, they are essential for appropriate physiological functioning at low levels, especially in molecular and cellular pathways bear the responsibility for learning, memory and cognition (GauravSinghal, et al. 2014).A huge No. of studies showed that IL-1β has a role in the pathogenic pathways of a variety of acute and chronic neurological diseases, and suggest that IL-1βis the missing link between a potentially beneficial inflammatory response and harmful glutamate excitotoxicity and oxidative stress (Fogal and Hewett

2008),In the normal brain, the levels of IL-1β are very low but its levels are elevated after several states such as damage and peripheral inflammation, inducedcentralIL-1β is largely produced by microglia but also by neurons(María, et al. 2013). The primary function of chemokines, a group of secreted proteins in the cytokine family, is to promote cell migration. Cytokines and chemokines are important for the brain's immunological function, as they aid in immunological surveillance, leukocyte trafficking, and employ other inflammatory factors. Immune cells and CNS cells can release cytokines and chemokines, in addition, respond to them via cytokine and chemokine receptors, during neuroinflammation (Marika, Salvatore and Rosalia 2020).

1.6.4.2. Transcriptional Factor NF-κB in Parkinson disease

NFκB firstly described by David Baltimore's group, is really important in the inflammatory response via controlling the expression of genes that code for inflammatory cytokines(IL-1β, TNFα), chemokines (IL-8) and nitric oxide production (iNOS). It is a "master switch" for inflammatory gene expression(Flood, et al. 2011).NF-κB, is believed to play a role in as a key regulator of a myriad of cellular processes that involved in several pathophysiologic pathways including misfolded SNCA aggregation associated with PD. SNCA accumulation, a hallmark of PD, is activated NF-κB in neurons, causing apoptosis to spread in a variety of ways. Furthermore, misfolded –SNCA produced by degenerating neurons stimulate various signaling pathways in glial cells, culminating in NF-κB stimulation and the generation of pro-inflammatory cytokeines, exacerbating neurodegenerative processes. On the other hand, NF-κB activation, acting as a double-edged sword, can be necessary for survival of neurons(Dolatshahi, et al. 2021).So,

agents that block the activation of NF-κB are capable of inhibiting the two major inflammatory pathways in microglia: activation of oxidative stress and production of inflammatory mediators (Flood, et al. 2011).

1.6.5. Proteins Synthesis and Degradation in Parkinson disease

The scientific community has been concerned with the involvement of disordered protein metabolism in PD since protein aggregates were discovered in the brains of people with Parkinson disease at the beginning of the 20th century. Recent research views protein production as consisting of transcription, translation, and post-translational modification, and protein degradation as including proteasome, lysosome, and ER-dependent processes.Recent studies on this subject have shown the hereditary component of Parkinson's disease (PD) and the numerous ways in which it is indicative of defective protein metabolism, particularly with regard to SNCA homeostasis and the role of the lysosome in pathogenic processes. (Langston and Cookson 2020).

1.6.6.Strategies of PD Neuroprotective Targets

Realization the major methods that involved in the initiation and dissemination of the illness, pathophysiology is critical for conferring neuroprotection against PD as follow (Sarkar, Raymick and Imam 2016). Six of these strategies are mentioned focusing on the two that are adopted in this study.The first strategy is the oxidative stress and mitochondrial dysfunction whose goals for neuroprotection include:(a) suppressing agents of DA metabolism, such as MAO inhibitors and DA receptor agonists; (b) electron transport improvers, such as CoQ10; (c)GSH promoters, such as selenium; (d) inhibitors of SNCA aggregation; (e) therapeutic compounds that decrease

SNCA protein levels; (f) enhancers of parkin function; and (g) instigaters of proteosomal or lysosomal mechanisms. The second strategy is neuroinflammation. The other strategies are protein aggregation and misfolding; excitotoxicity; apoptosis and cell death pathways; and loss of trophic factor.

1.7. Diagnosis of Parkinson disease

Parkinson disease remains a clinical diagnosis. The asymmetric symptoms of resting tremor, bradykinesia, and rigidity with favorable response to dopaminergic therapy suggest its diagnosis.(Theresa A., FAAN2019). Exclusionary features may include severe dysautonomia, early hallucinations, dementia preceding motor symptoms, and postural instability and freezing within the first 3 years after diagnosis (Berg D, Lang2013) The UK Parkinson's Disease Society Brain Bank Diagnostic Criteria 28 are listed below: Clinical Diagnostic Standards for the Brain Bank of the UK Parkinson's Disease Society:

step -1-: identify Parkinson's syndrome in patient

Bradykinesia, which is characterized by a progressive slowing down of the speed and magnitude of repetitive movements, at least one of the following conditions: Stiffness of the muscles, postural instability and 4-6Hz resting tremor, Not happened due to primary visual, vestibular, cerebellar,orproprioceptiveimpairment.

Step -2-: Excluded the characteristics of Parkinson Disease

check to see if there history of several strokes with progressive parkinsonian symptoms, previous recurrent head injuries ,definite encephalitis in the past, sight crises, when symptoms first appear, neuroleptics at the beginning of symptoms, persistent remission, after three years, strictly unilateral traits, Cerebellar signs, Early severe autonomic involvement, Early severe dementia with disturbances of memory, praxis, and language, Babinski sign, Supranuclear gaze palsy, MPTP exposure, Presence of a cerebral tumor or communicating hydrocephalus on CT scan, Negative response to large doses of levodopa (if malabsorption excluded)

Step -3-: Supportive Prospective Positive Criteria for Parkinson Disease

If three or more symptoms are required for a Parkinson disease diagnosis, unidirectional onset, current resting tremor, advancing disorder, persistent asymmetry most noticeable on the side of onset, Excellent (70-100%) response to levodopa, severe levodopa- induced chorea, responsiveness to levodopa for at least five years, Ten years or more of clinical experience.

1.8. Pharmacological Treatment of Parkinson disease

Parkinson disease (PD) diagnosis usually depends on motor symptoms; thus, anyone who has tremors, stiffness, sluggishness, balance problems, or gait difficulties should be suspected. Use of CT or MRI brain scanning is very helpful in diagnosing PD but should not be routinely used according to the Canadian Guideline. There are no treatments that can delay or stop brain degeneration in people with PD. A clear response to dopamine replacement medication (e.g., levodopa/ carbidopa 600 mg/d) in an individual with PD could help to reinforce that an accurate diagnosis has been established

(Grimes, et al. 2019).PD has no cure and nothing is known to modify the progression of the disease and current treatment options provide only symptomatic relief(Ellis and Fell 2017). Drugs used to relief PD symptoms are:

1.Levodopa**.**

2.Dopamine Receptor Agonists(Bromocriptine, Pergolide, Pramipexole, Ropinirole andRotigotine).

3.Monoamine Oxidase Inhibitors which inhibit break down of dopamine (Selegiline and Rasagiline).

4.Catechol-O-Methyltrasferase Inhibitors(Tolcapone and Entacapone).

5. Apomorphine.

6. Amantadine(binds and blocks NMDA glutamate recepters).

7. Acetylcholine-Blocking drugs(Benztropine, Biperiden, Orphenadrine, procyclidine and Trihexyphenidyl)(Katzung 2018).

1.8.1. Dopamine agonists

Dopamine agonists (DAs) are a class of medications having a preference for the D2-receptor subfamily of dopamine receptors that directly stimulate striatal dopamine receptors to achieve their antiparkinsonian effects.The ergoline compound bromocriptine was the first member of this class to be introduced, along with a number of additional ergoline DAs. (Pramipexol, Ropinirol, Rotigotine, Apomorphine)

1.8.2. Enzyme inhibitors

Early on, it was believed that one way to improve the effectiveness of levodopa treatment was to block the metabolism of central dopamine by inhibiting monoamine-oxidase (MAO).It had to be terminated since the nonselective MAO inhibitors that were then on the market had a risk of causing hypertensive crisis after eating foods high in tyramine (the "cheese effect") due to central serotonergic overactivity. The introduction of MAO inhibition into clinical practice in PD was made possible by the formation of selective inhibitors of the B-type of the enzyme, which mostly metabolizes dopamine.The irreversible enzyme blockers selegiline and rasagiline as well as the reversible inhibitor safinamide, which also has extra indirect glutamatergic activity, are being used as MAO-B inhibitors. (Huot P, Fox SH2015) They have a weaker antiparkinsonian impact than levodopa or DAs.

1.8.3. Pharmacological Side Effects of Other Parkinson's Drugs

Drugs that used to treat PD other than L-Dopa are stated below with their side effects:

1)Dopamine agonists: Orthostatic hypotension, hallucinations, nausea, impulse control disorders, increased sleepiness including sleep attacks and edema

2)MAO-BIs: Dizziness, headache, confusion, exacerbation of L-Dopa adverse effects, arthralgia, dyspepsia, depression and flulike syndrome.

3)COMT-Is: Dark-colored urine, exacerbation of levodopa adverse effects and hepatotoxicity.

4)Amantadine : Hallucinations, confusion, blurred vision, ankle edema, livedo reticularis, nausea, dry mouth and constipation.

5)Anticholinergic : Hallucinations, cognitive impairment, nausea, dry mouth, blurred vision, urinary retention and constipation(Connolly and Lang 2014).

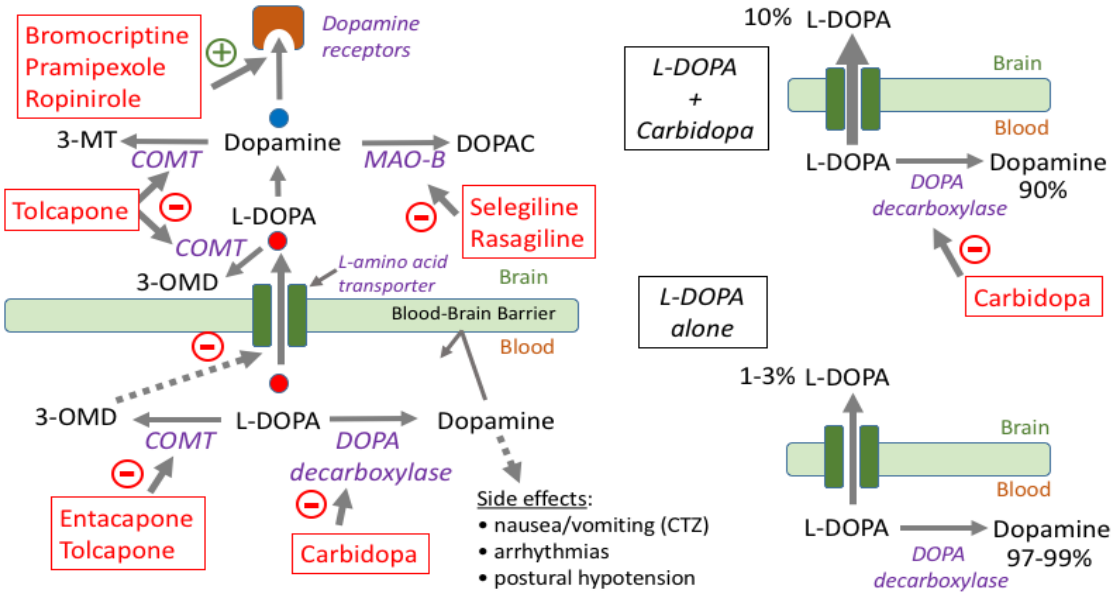


Figure 1.7.: pharmacological treatment of Parkinson disease (Katzung BG, Masters SB2015)

1.8.4. Levodopa

Levodopa, which unlike dopamine is able to pass the blood-brain barrier, is the aromatic amino acid precursor of the hormone dopamine. When the enzyme central aromatic amino acid decarboxylase (AADC) is activated, levodopa is transformed into dopamine.This process happens in the remaining nigrostriatal projection neurons and most likely involves the presynaptic storage and release of the neurotransmitter. It also probably happens ectopically in serotonergic neurons, which could result in an uncontrolled release of synaptic dopamine in the striatum. (Rylander D, Parent M2010)

Levodopa has been proven to be highly effective in reducing the motor symptoms of Parkinson's disease (PD) in numerous randomized controlled trials since it was originally demonstrated in the 1960s. (Fox SH, Katzenschlager R, L2018)(LeWitt PA, Fahn S.2016) Until recently, no medication—possibly with the exception of the dopamine agonist apomorphine—has been discovered to have an equivalent symptomatic effect size to levodopa. The D1-like receptor (D1R and D5R) and the D2-like receptor (D2R, D3R and D4R) are the two subclasses of DA-ergic receptors that have been discovered in the brain .The GABAergic medium spiny neurons in the dorsal striatum express D1R and D2R.While the D2R contributes to the indirect pathways, the D1R is involved in the direct pathway's information flow.The basic symptoms of PD are caused by their reconfiguration in the basal ganglia, which includes increased D2R and decreased D1R expression as well as the loss of presynaptic D2R. (Muthuraman, et al. 2018).When symptoms of PD become difficult to treat with other antiparkinsonian drugs, levodopa is usually administered. Recent evidence suggests that L-Dopa can help halt the progression of PD(Gandhi and Saadabadi 2020).

1.8.4.1. Biochemistry of L-Dopa

Dopamine's precursor, which is L-Dopa, is an aromatic amino acid with a molecular formula of C9H11NO4 (Figure 1.8). It is a pro-drug that is converted to dopamine by DOPA decarboxylase and can cross the BBB. It is the most effective and widely used medication for PD. Carbidopa, an inhibiter of L-amino acid decarboxylase, the plasma enzyme that metabolizes L-Dopa peripherally, is commonly taken with L-Dopa. Various metabolic pathways convert L-Dopa to DA, which is then transformed to sulfated or

glucuronidated metabolites, epinephrine E, or HAV. DOPAC(13-47 %) and HAV (23-39 %) are the main metabolites (Ncbi 2019).

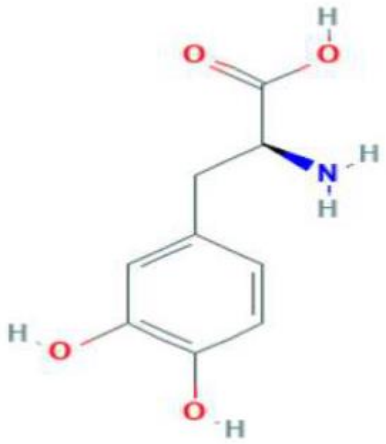


Figure1.8: chemical structure of levodopa

1.8.4.2. Managing Motor Fluctuations

The More serious and persistent effects of levodopa, such as ‘‘wearing off" fluctuations and dyskinesias (abnormal involuntary movements), arise when PD develops (or prolong-term use of L-Dopa); these motor difficulties impact 75% of patients after six years of L-Dopa medication. These issues have a significant impact on the magnificence of life and motor status of patients, and they are difficult not only for the patient but also for the physician who is treating (Trenkwalder, et al. 2019).Managing recurrence of symptoms between drug doses increase the dosage of DA-ergic medication, add another DA-ergic medication, divide the L-Dopa dosage into smaller and more divided doses (L-Dopadose fragmentation), or add COMT inhibitors or MAO-B inhibitors to block the breakdown of L-Dopa and DA(Connolly and Lang 2014).

1.8.4.3. Forms of Carbidopa/ levodopa

There is many different forms of Carbidopa/ L-Dopa according to Parkinsons Foundation in March/2021 (Parkinsons Foundation 2021)which include: Immediate Release, Controlled Release, orally disintegrating tablets (Parcopa), enteral Suspension (Duopa), extended releasing capsules (Rytary), entacapone tablets (Stalevo).In 2018, an inhaled formulation of L-Dopa as an additional therapy to L-Dopa/ carbidopa for the treatment of PD where approved by the Food and Drug Administration (FDA) (Gandhi and Saadabadi 2020.).

1.8.4.4. Side effects of L-Dopa

Postural hypotension, headache, Dizziness, nausea, somnolence, and are the most prevalent side effects of L-Dopa. Carbidopa intake should be increased to alleviate nausea(Connolly and Lang 2014).Elderly people must be treated with extra attention since they may be more vulnerable to the effects of the CNS. Confusion, hallucinations, psychosis, and agitation are the most common side effects in elderly people on L-Dopa (Trenkwalder,et al. 2019).Dizziness and postural hypotension are the most prevalent cardiovascular side effects; Thus some patients may need to reduce or stop taking their antihypertensive drugs. In investigations, researchers also report cardiac arrhythmias. Because the onset is sudden and without notice, vigilance is required when operating motor vehicles(Kalinderi, Papaliagkas and Fidani 2019).

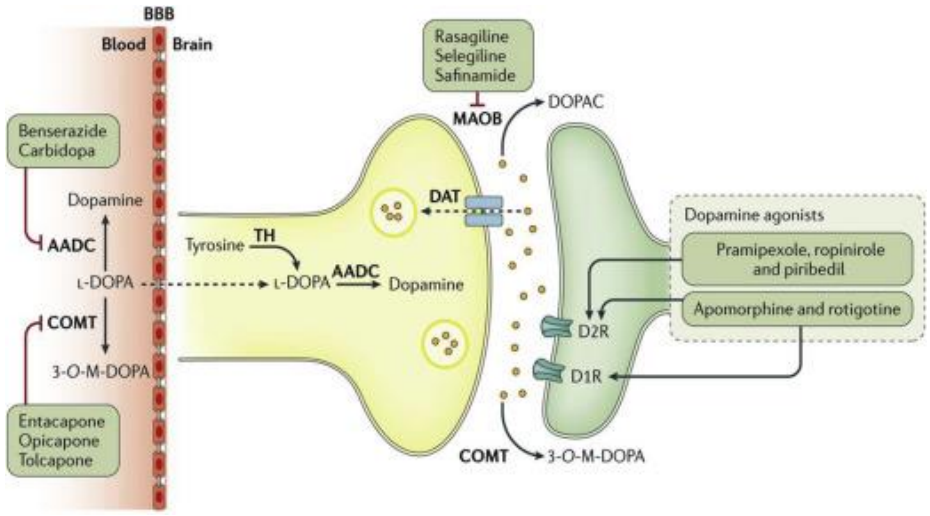


Figure1.9: The Scheme represent the nigrostriatal dopamine synapse. (FromPoewe W, Seppi K, Tanner CM, et al. Parkinson disease.NatRev Dis Prim. 2017).

1.9. Models of Parkinson disease

Usually Disease animal models are important for drug discovery. An ideal PD animal model would have all of the clinical and pathologic features of human PD. However, no current PD animal model can replicate all of the behavioral and pathologic symptoms seen in human PD (Prasad and Hung 2020). Motor abnormalities, gradual loss of DA-ergic neuronal cells in the SNpc, and LBs(Lewy bodies) have all been linked to specific neurotoxins in studies. In spite of the effectiveness of currently available remedies in these neurotoxins induced animal models does not translate well to in a therapeutic setting, we believe that neurotoxin-induced PD animal models will eventually lead to successful PD therapy(Zeng, et al. 2018).

The fact that samples of the primary organ affected by PD, the brain, can only be studied post-mortem creates challenges for researchers trying to understand

the early biochemical changes of the disease.This restriction has prompted the creation of numerous animal models of PD, which can be broadly divided into the following categories:

1. Toxin model induced by rotenone , MPTP, and 6-OHDA.

2. models of inflammation brought on by LPS.

3. Drug-induced models brought on by reserpine and haloparidol. (Johnson and Bobrovskaya 2014).

4. models of genetic change brought on by genetic alterations of SNCA, PINK1, DJ-1, LRRK2(Konnova and Swanberg .2018).Animal models have shown to be essential tools for evaluating novel possible symptomatic, neuroprotective, and neurorestorative therapy as well as studying PD pathogenesis pathways to improve understanding of the condition (Johnson and Bobrovskaya 2014).

1.9.1. Neurotoxins in Vivo Models of Parkinson disease

By The use of the pharmacologically induced animal models of Parkinson is important in the development of treatments. There are a number of neurotoxins that are structurally similar to catecholamine, DA, and noradrenaline. The recent neurotoxins applied to induce the PD are 6-OHDA, MPTP, PQ, rotenone, and permethrin. Different types of lesioning and induction procedures are used to create PD animal models. Each neurotoxic has its own set of damaging mechanisms that cause neurodegeneration in the brain (Prasad and Hung 2020)

The exact mechanisms by which these neurotoxin can pass across the BBB are unknown. Because rotenone is very lipophilic, it is expected to cross,

while PQ is thought to act by using neutral amino acid and sodium-dependent transporters. With the possible exception of PQ, all compounds target the mitochondrial complex. Rotenone particularly targets complex I, while Maneb targets complex III, and PQ-induced mitochondrial dysfunction could be caused by redox cycling in the cytosol. The compound's effects with respiratory cells is particularly harmful, and it eventually leads to cell death(Cicchetti, Drouin-Ouellet and Gross 2009) and(Hirsch and Hunot 2009).

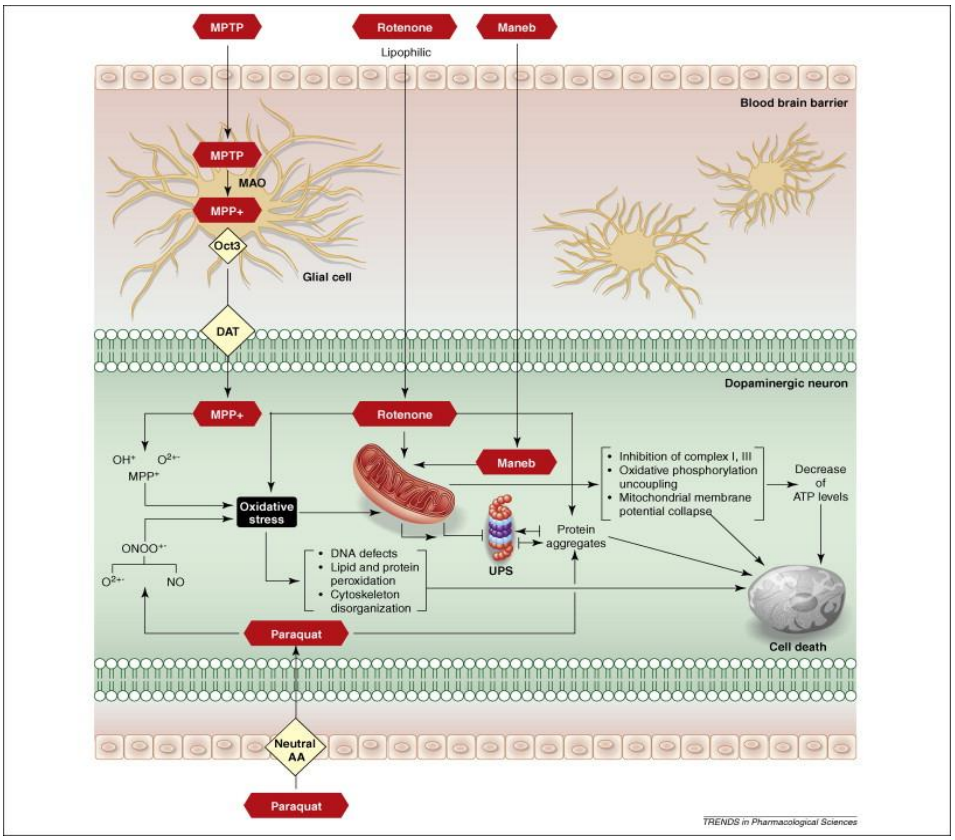


Figure 1.10: Mechanisms of actions of various toxins used to produce PD models. (Robert E. Gross2009)

1.9.2. Rotenone

Rotenone is a neurotoxin that used to make the most firmly established PD models, acted by inhibiting of mitochondrial complex I, In the 1980s, rotenone was first employed in PD research. (Heikkila,et al. 1985). Natural pesticide and insecticide rotenone is derived from the roots of plants of the genera Lonchoccarpus and Deriss. (Soloway 1976). Rotenone has been used as an insecticide in vegetable crops and to control nuisance fish populations, despite recent research linking it to an increased risk of Parkinson's disease (PD).Due to rotenone's high lipophilicity, which allows it to easily traverse all biological membranes including the BBB, so cells do not need transporters to take it into them. (Greenamyre and Timothy 2012).Selective SN degeneration, SNCA accumulation and aggregation, oxidative stress, neuroinflammation, microglial stimulation, impaired locomotor function, and disruption of mitochondrial complex I are all observed, resulting in pathology that is firmly similar to that seen in human PD (Javed1,et al. 2016). Rotenone block complex I in the electron transport chain in the mitochondria, resulting in lower ATP generation, which can result in ROS such superoxide, lowering GSH and causing oxidative stress(Duty and Jenner, 2011) and (Martinez and Greenamyre, 2012).Rotenone-induced PD models occurred through progressive destruction of DA-ergic neurons and the development of LBs in the SN simulating experimental aspects of idiopathic PD. For developing PD models in rats, many exposure routes like oral, subcutaneous (Sc), osmotic pumps and IP routes are used. The postmortem studies provided more good proof for the role of hyperoxidation and protein carbonyl formation in the etiology of rotenone-induced PD (Prasad and Hung 2020).

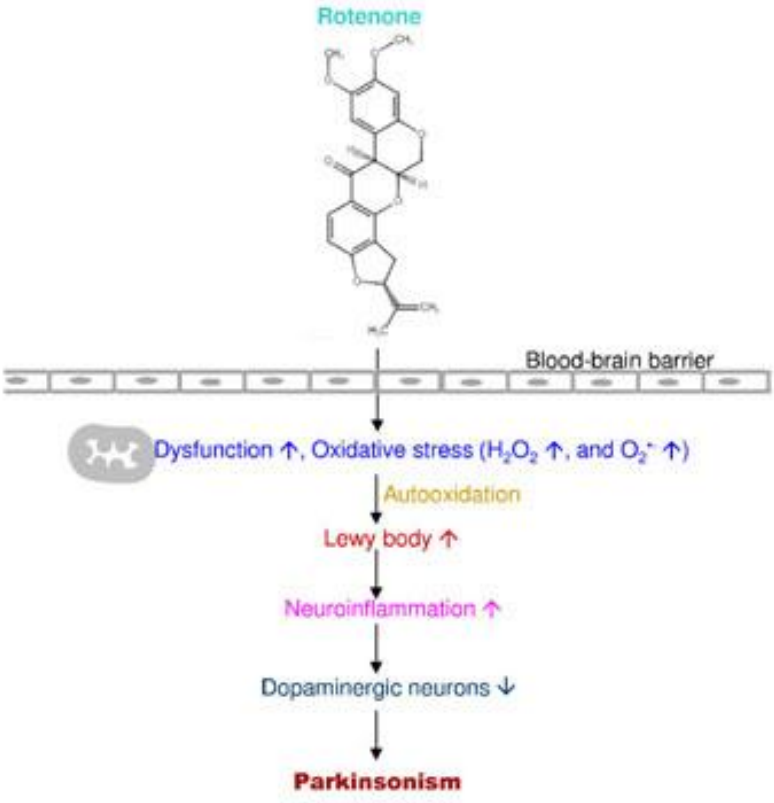


Figure 7. Putative mechanism of action of rotenone in PD progression.

Figure 1.11: Mechanism of Action of Rotenone in PD pathogenesis

The rat rotenone model has a number of flaws, including a loss of consistency in the number of animals that acquire DA-ergic nigrostriatal lesions, the location of the lesions, the size of the lesions, and the death rate (Johnson and Bobrovskaya 2014).

1.10. Salvia officinalis

Salvia officinalis (Sage) is a plant in the family of Labiatae /Lamiaceae. It is native to Middle East and Mediterranean areas, but today has been distributed throughout the world. (.Bisset NG, Wichtl M.2001) In folk medicine, S. officinalis has been used for the treatment of different kinds of disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycemia. (5.Garcia CSC,

Menti C2016) In traditional medicine of Europe, S. officinalis has been used to treat mild dyspepsia (including bloating and heartburn), increased sweating, age-related cognitive impairments, and skin and throat inflammations. European Medicines Agency.2009 There are many different pharmacological properties for S. officinalis include: anticancer, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycemic, and hypolipidemic effects.

1.10.1. Bioactive ingredients of Salvia Officinalis

The main phytochemicals found in S. officinalis' flowers, leaves, and stem are well known.S. officinalis contains a variety of different constituents, such as alkaloids, fatty acids, carbohydrates, glycosidic derivatives (such as flavonoid glycosides, cardiac glycosides, and saponins), phenolic compounds (such as tannins, flavonoids, and coumarins,), polyacetylenes, steroids, terpenes/terpenoids (such as monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids).(Wang M, Kikuzaki H, 2000) (Badiee P, Nasirzadeh AR,2012). The majority of S. officinalis' reported phytochemicals have been obtained from the plant's essential oil, aqueous extract, alcoholic extract, butanol fraction, and infusion preparation. The essential oil made from S. officinalis aerial parts has more than 120 components that have been identified. The main components of the oil include borneol, camphor, humulene, caryophyllene, elemene ,cineole, thujone, pinene, and ledene (Hayouni EA, Chraief I2008) . Rosmarinic acid and luteolin-7-glucoside are two flavonoids abundant in S. Officinalis' alcoholic and aqueous preparations. The methanolic extract of S. officinalis also contains phenolic acids such caffeic acid and 3-Caffeoylquinic acid.Infusion made from S. officinalis has been found to contain several flavonoids, including chlorogenic acid, ellagic

acid, epicatecin, epigallocatechin gallate, quercetin, rosmarinic acid, rutin, and luteolin-7-glucoside, as well as several volatile substances, including borneol, cineole, camphor, and thujone. (.Hernandez-Saavedra D,2016) (.Lima CF, Andrade PB2005) The most prevalent flavonoids in the S. officinalis infusion extract are rosmarinic acid and ellagic acid, followed by rutin, chlorogenic acid, and quercetin..(.Hernandez-Saavedra D,2016) In this plant, arabinose, galactose, glucose, mannose, xylose, uronic acids, and rhamnose are the carbohydrates that are characterized as being most abundant. (Capek P, Hríbalova V.2004) Linalool is the most prevalent phytochemical in the stem of S. officinalis, while a-pinene and cineole are the most abundant phytochemicals in the flowers, and bornyl acetate, camphene, camphor, humulene, limonene, and thujone are the most prevalent phytochemicals in the leaves. (Velickovic DT, RanCelovic NV,2003) But it should be remembered that, like to other herbs, S. Officinalis' chemical nature will vary according on environmental factors like temperature, water availability, and height..(Russo A, Formisano C,2013)

1.10.2. Antioxidant activities of salvia officinalis

Numerous diseases, including diabetes, cardiovascular conditions, cancer, and neurological problems, are caused by and exacerbated oxidative stress. Increased oxidative stress happens when the capacity of antioxidant defenses like catalase, glutathione peroxidase, and superoxide dismutase activities is exceeded by the generation of ROS by the mitochondrial electron transport chain, NADPH oxidase, uncoupled nitric oxide syntheses, and xanthine oxidase.Natural antioxidants helps protect cells from the excessive generation of ROS and can consequently minimize tissue damage brought on by oxidative stress. Multiple investigations have found S. officinalis possess

strong antioxidant properties.Rat hepatocytes are more resistant to oxidative stress when rats' drinking water is enriched with S. officinalis extract.By increasing glutathione peroxidase activity, it serves to protect hepatocytes from DNA damage brought on by dimethoxy naphthoquinone and hydrogen peroxide. rosmarinic acid, Carnosol, and carnosic acid are the components of S. officinalis that are the most potent antioxidants, followed by caffeic acid, rosmanol, genkwanin, rosmadial, and cirsimaritin. Carnosol has similar radical scavenging properties as a-tocopherol. The rosmarinic acid derivatives have a 15-20 times greater superoxide scavenging activity than trolox, a synthetic water-soluble vitamin E. Rosmarinic acid improves the activity of pancreatic catalase, glutathione-S-transferase, glutathione peroxidase, and superoxide dismutase in streptozotocin-induced diabetic rats. Other S. Officinalis flavonoids, in particular rutin, quercetin and exhibit potent antioxidant properties in addition to rosmarinic acid.

1.10.3. Anti-inflammatory and anti nociceptive activities

The two most significant symptoms that follow tissue injury are pain and inflammation.The pharmaceutical management of these symptoms still includes non-steroidal anti-inflammatory medications as a critical component.However, these medications come with unfavorable side effects include digestive and cardiovascular issues when used in clinical settings. Therefore, the study of newer anti-inflammatory and antinociceptive substances with minimal undesirable effects continues to be an interesting topic. S. officinalis has been proven in pharmacological research to have anti-inflammatory and antinociceptive properties. For instance, it has been demonstrated that this plant aids in the management of neuropathic pain in peripheral neuropathy caused by chemotherapy. The chloroform extract of S.

officinalis has greater anti-inflammatory activity compared to the methanolic extract and essential oil. The substances that are most likely responsible for the herb's anti-inflammatory and anti-nociceptive effects are flavonoids and terpenes.

1.10.4. Toxicological studies

Clinical studies have shown that consuming S. officinalis does not have any serious adverse effects. However, ethanolic extract and volatile oil from S. officinalis (equivalent to more than 15 g of the leaves) may cause several undesirable side effects, including tachycardia, vomiting, salivation, cyanosis, vertigo, hot flashes, tongue swallowing, allergic responses and even convulsion. Because S. officinalis oil directly affects the neurological system (at levels more than 0.5 g/kg), it has a pro-convulsant effect.

In S. officinalis, camphor, thujone, and terpene ketones are thought to be the most hazardous elements. The fetus and newborn may experience harmful consequences from these substances. Therefore, it is not advised to consume S. Officinalis when pregnant or nursing. The LD50 of S. officinalis oil (when taken orally) and the methanolic extract (when injected intraperitoneally) have been determined to be 2.6 g/kg and 4 g/kg, respectively, according to results from animal investigations. S. officinalis tea has been found to increase the CCl4-induced hepatotoxicity in mice. However, no hepatotoxic side effects were noted in clinical studies.

1.11.The Behavioral Tests of Parkinson disease

1.11.1. The Rotarod Test

All animals' motor coordination and performance were estimated using a rotarod apparatus. The rotarod apparatus is made up of a rotating rod with a diameter of 75 mm and a height of 40 cm that is separated into four sections (Rustay, Wahlsten and Crabbe 2003). Prior to the protocol, all of the animals had been trained. The animals were placed on the spinning rod at 20 rpm on the day after the end of the experiment to measure their motor coordination, and the average fall-off time was recorded (Sharma, Raj and Singh 2019).

1. 11. 2. The Open Field Test

The open-field exam essessed spontaneous locomotor activity and performance. A wooden, rectangular, light brown–colored open-field device measuring 100 x 100 x 40 cm3 makes up the open-field apparatus (Kumar, Kalonia and Kumar 2012), consisting of a square floor divided by thin white lines into 100 equal squares. The activity of each rat was measured for about 10 minutes by placing the rat in the center of the apparatus. Crossings and rearing behaviors are used to assess hyperactivity in the open-field apparatus. The total number of square crossings throughout the test period is referred to as crossings, and it is used to determine the animals' locomotor activity. During the test period, the total number of erect postures adopted by the rodent with the intent of exploring is referred to as rearing. The total number of visits to the center of the open-field is used to assess risk-factor behavior. The term grooming refers to the overall amount of time spent grooming (Figure 1.12) (Martinez-Gonzalez, et al. 2004).

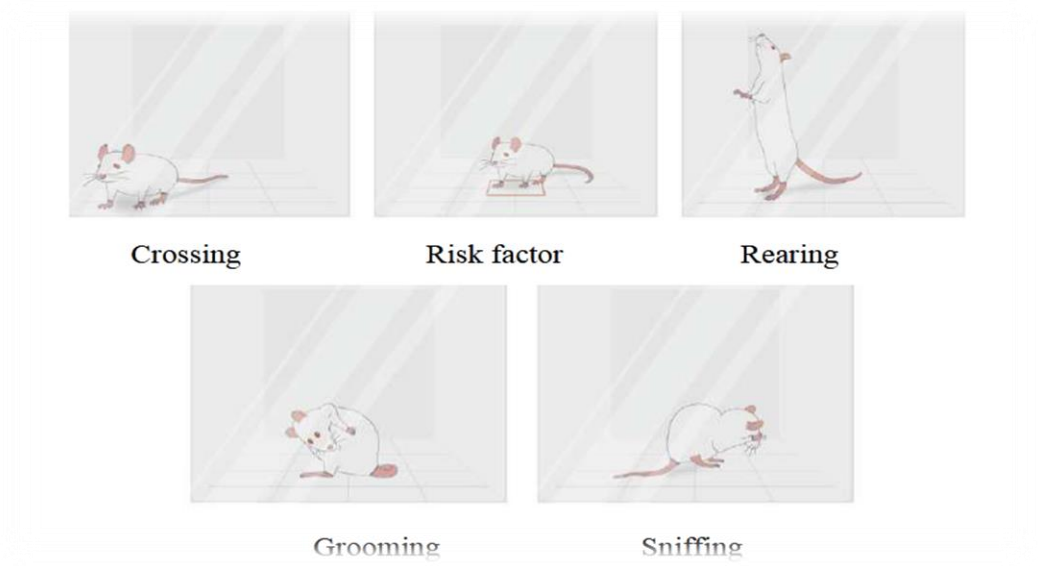


Figure 1.12: Open Field Test Representation. Crossing, Risk factor, Rearing, Grooming, Sniffing.

1. 11. 3. The latency of falling time Test

the Rats were suspended by their forelimbs from a steel rod (60 cm long, 2cm in diameter) hanging 60 cm above the bench to assess motor strength and muscular rigidity. Each rat was exposed to the apparatus three times every trial, and the time until the rat completely releases its grasp and falls down was recorded (Abdelkadera, Faridb, et al. 2020).

Chapter Two

Materials and methods

2. 1. Materials

2. 1. 1. Animals

One hundred healthy adult Albino rats weighing between (150-300g). The College of Medicine/ University of Babylon's Animal House served as the residence for the rats. They were housed in 20 cages, five rats in each cage, at a temperature of 25 centigrade, with a cycle of fourteen hours of sunshine and ten hours of night, as well as unlimited access to food and water. After two weeks of adaption, the animals were placed into five groups at random; with 10 rats in each group.The study was conducted at the College of Medicine/ University of Babylon, from September 2021 to March 2022.

2. 1. 2. Instruments and equipment

The instruments and equipment which were employed in this study with their suppliers are listed in Table 2.1.

Table 2.1: Instruments and equipment of the study

No.	Instrument/ Equipment	Company/ Country
1.	Automatic tissue processor	Bio Optica, Italy
2.	Centrifuge	Hitachi, Germany
3.	Digital rotarod	Bionec Mobin/ Iran

4.	Electronic scale	Sensor Disk Technology/ China
5.	Elisa (reader/ washer/ printer)	Haman/ Germany
6.	Eppendorf plastic tubes	Sun/ Jordan
7.	High speed cold centrifuge	Eppendorf/ Germany
8.	Hot and cold plate	Nanjing, China
9.	Homogenizer drill	China
10.	Incubator	Memmert/ Germany
11.	Microscope	Leica, Germany
12.	Micropipettes (different volumes)	Eppendorf/ Germany
13.	Oven	Labcon/ Germany
14.	Plastic cassette for tissue processing	Anveon technologies, India
15.	Positive charged slides	Bio SB, U.S.A.
16.	Qualitative filter paper	Jiao Jie/ China
17.	Refrigerator	Concord/ Lebanon
18.	Rotary evaporator	Laborota/Germany
19.	Sensitive balance	Sartorius/ Germany
20.	Shaker	Gyro-Rocker/UK
21.	Spectrophotometer	Jenway/ England
22.	Surgical set	China
23.	Video camera	Samsung/ Korea
24.	Water bath	Polyscience/ USA

2. 2. Chemicals

The chemicals that are used in this study are listed in Table 2.2.

Table 2.2: The chemicals used in this study

No.	Chemicals	Company/ Country
1.	Absolute Ethanol solution	Scharlan/ Spain
2.	Diethyl ether solution	Laboratory reagents, India
3.	Dimethyl sulfoxide solution	Chem Lab NV/ Belgium
4.	Eosin stain	Sigma/ U.S.A
5.	Formaline solution	Laboratory reagents, India
6.	Hematoxyline stain	Thermo Shandon/ U.S.
7.	Olive oil	Cesar Spain
8.	Phosphate buffer saline	Hi Media Lab/ India
9.	Rotenone Power vial	Med Chem Express/ USA
10.	Sinemet tab	MSD/ USA

2. 3. Kits

The kits that are used in this study are listed in Table 2.3.

NO	Kit	Company/ country
1.	**Rat MDA ELISA Kit**	BT.LAB/CHINA
	Standard solution12.5 nmol/ml 0.5ml*1	
	Pre-coated ELISAplate 12*8 well strips*1	
	Standard diluent 3ml*1	
	Streptavidin – HRP6ml *1	
	Stop solution 6ml *1	
	Substrate solution A 6ml *1	
	Substrate solution B 6ml *1	
	Wash buffer concentrate (25x) 20 ml *1	
	Biotinylated rat MDA Antibody 1 ml *1	
2.	**Rat tyrosine hydroxylase ELISA kit**	Elab science bilechnology USA
	3%H2O2 3ml *1	
	polymer helper 3ml*1	
	Polyperoxidase-anti-mouse/ Rabbit IgG 3ml*1	
	Normal Goat serum 3ml*1	
	DAB concentrate 150ml*1	
	DAB substrate 3ml*1	

2.4. Behavioral Equipment

2.4.1. Rotarod Apparatus

The study examined rodents' physical capacity and coordination with a digital rotarod test. Rats should balance on a rotating cylinder with a variable speed for it to work. (Rozas, Guerra and Labandeira-García 1997). Each rat was placed on the 20 rpm rotating cylinder and studied for three minutes. Rat performance for motor coordination was graded depending on the number of spins. After each test, a 10 % ethanol solution was used to clean the instrument. (Rao, et al. 2019).

A B

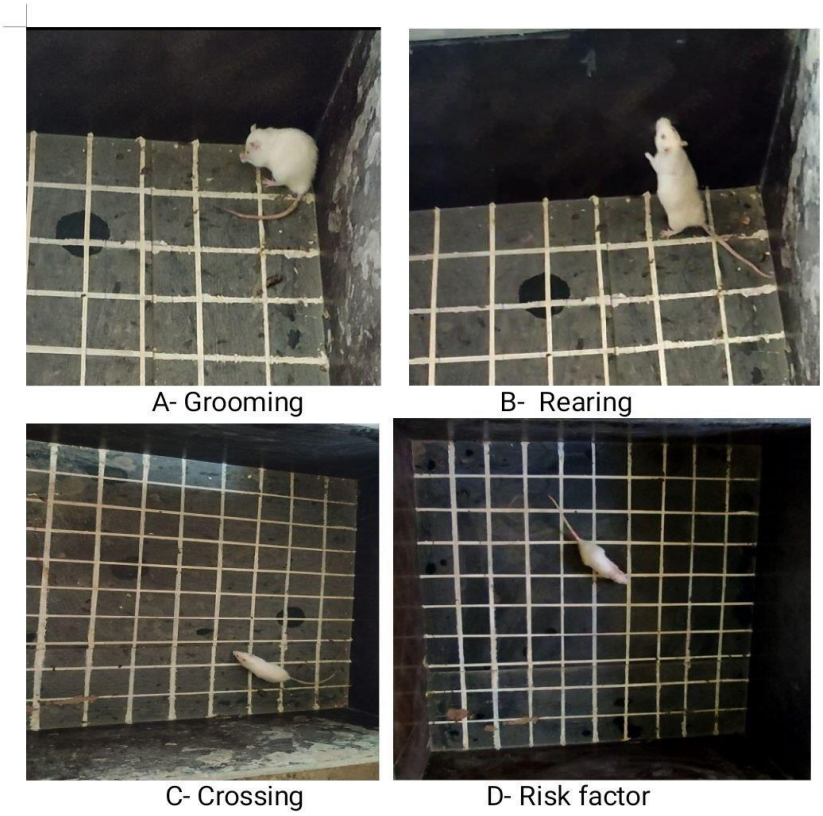
Picture2.1: A-Digital rotarod device B- Roterod test

2. 4. 2. Open Field Box

This wooden box (100 x 100cm) was made by researcher according to (Martinez-Gonzalez, et al.2004) consisting of a square floor divided by thin white lines into 100 equal squares. The activity of each rat was measured for about 10 minutes by placing the rat in the center of the apparatus (Figure2.2). Crossings and rearing behaviors are used to assess hyperactivity in the open-field apparatus. The total no. of squares crossing throughout the test time is

referred to as crossings, and it is used to determine the animals' locomotor activity. During the test period, the total no. of erect postures exhibited by the rat with the intent of exploring is referred to as rearing. The total No. of visits to the center of the open field is used to assess risk-taking behavior. The term grooming refers to the overall amount of period spent grooming (Figure2.3). All behaviors were recorded by video camera

Picture 2.2: Open field box



Picture 2.3: Representation of A- Grooming, B- Rearing, C- crossing , D- Risk factor.

2. 4. 3. Force Griping Apparatus

A wooden chamber containing a stainless beam (1m x 60cm x60cm) was built by researcher (Figure2.4). The rat was placed on beam and the latency time to fall was recorded by video camera (Abdelkadera, Faridb, et al. 2020).

Picture 2.4: Force Griping Test

2.5. The Pilot Study

The purpose of the pilot study is to choose the appropriate dose and route of rotenone. Thus, sixteen adult, Albino, male rats were enrolled in the pilot study which were randomly divided into four groups, four rats in each group.

•Group I is the negative control

•Group II: each rat was received1.5mg/kg of rotenone by subcutaneous route every other day for 21 days.

•Group III: each rat was received 2.5mg/kg of rotenone by IP route every other day for 21 days.

•Group IV: each rat was received 2mg/kg of rotenone by IP route every day for 21 days. The results showed that highest mortality rate occurred in group II and the clearest symptoms of parkinsonism occurred in group III.

2. 6. Preparation of plant

The dried leaves and roots of Salvia officinalis, Figure 2 have been confirmed to be S. officinalis by College of Agriculture/ Medicinal Plant Department/ Al-Qasim Green University according to document No.1067 /2022

Picture2.5: Dried leaves and roots of Salvia officinalis.

Infusion of sage leaves, or so-called production of sage tea, is a very popular preparation in folk medicine. According to (Radulescu, V.; Chiliment, S.; Oprea, E. C2004), 100 mL of boiling water are poured over 5 g of leaves of Salvia officinalis L. and filtered after 30min.increasing the extraction temperature from 25 ◦C to 80 ◦C caused extracts yielded with higher phenolic content. These results were explained by (Dent, M.2013) who also studied sage aqueous extracts, reporting that the mass fraction of total polyphenols significantly depends on the extraction temperature (Dent, M.; Dragovi´c-Uzelac 2013). The highest total phenolic content and maximum antioxidant capacity of aqueous extracts of sage at temperatures were achieved at 80 ◦C. (Nefeli S. Sotiropoulou. 2020).

2.7. Preparation of Rotenone

To cause Parkinsonism, rats were intraperitoneally injected rotenone (2.5 mg/kg BW). 125 mg of rotenone were initially dissolved in 1 mL of a 50X stock solution of dimethyl sulfoxide (DMSO) (Javed, et al. 2016). A fresh solution was made twice a week, and the stock solution was diluted in 1960 ml of olive oil with 40 ml of the stock solution. The solution was vortexed to create a homogenous mixture before being administered to the rat. In contrast to the control group of animals, which only received the vehicle (olive oil/DMSO), each rat received 1ml/kg of the produced solution. (Mbiydzenyuy, et al. 2018).

2.8. Sinemet Preparation

Sinemet (25/250 mg)tablet after crashing in water(Colpaert 1987), prepared daily in a dose of 10 mg/kg for each rat in group 3 with shake before administration(Priyanga, Vdayalakshmi and Selvaraj 2017).

2.9. Research Design

In random manner, the fifty rats were divided into five groups, ten animals in each group as follow:

•Group I: are healthy control group.

•and the other fourty rats were Induced with parkinsonism by rotenone IP 2.5mg/kg every 48hr (every other day) for 4 weeks (Alabi, et al. 2019) and subdivided as follow:

a) Group II: untreated Parkinsonian rats.

b) Group III: 10mg/kg of Sinemet tablet every day for 4 weeks orally by a gavage.

c) Group IV: 500mg/kg of Salvia officinalis every day for 4 weeks orally by a Gavage.

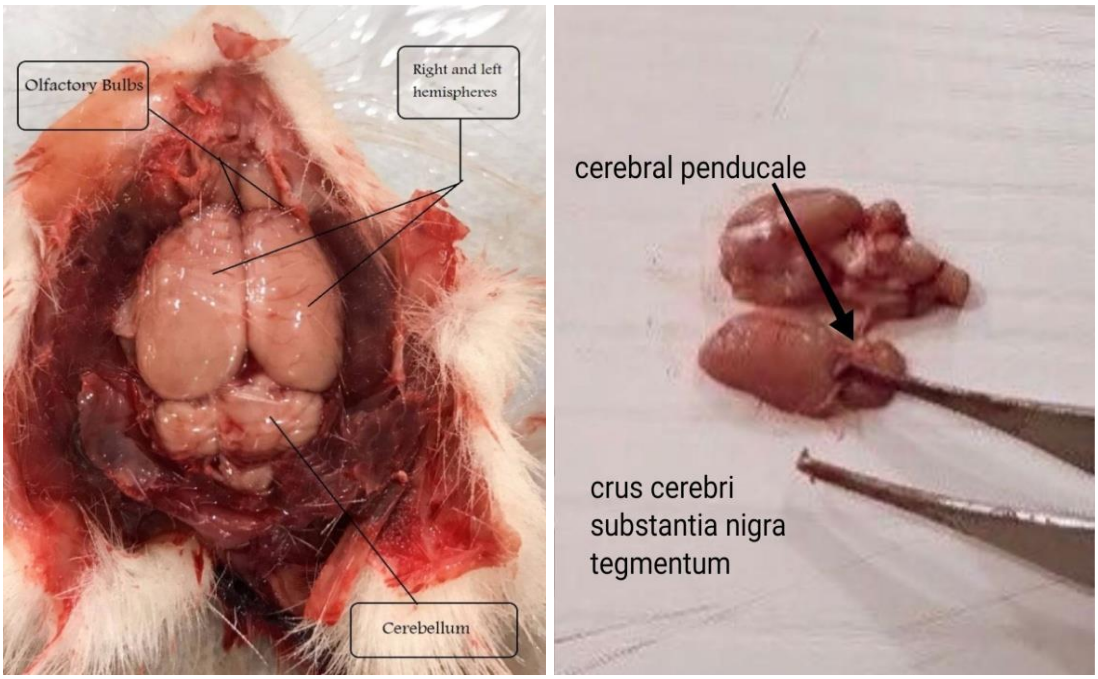
d)Group V: 500mg/kg of Salvia officinalis + 10mg/kg of Sinemet tablet every day for 4 weeks orally by a gavage .

•The rats were weighed in day 0, 15, and 30 of the experiment.

•24 hours after the last dose, on 29th Day behavioral assessments were conducted to compare the progression of parkinsonism and the efficacy of therapy. Each animal performed three trials on the rotarod, followed by three trials on the narrow beam. then in the open field for 10 min. and all behaviors were recorded by video camera.

2.9.1. Dissection of the Brain

Each rat was sacrificed on the 30th day, and the brains were extracted after the posterior foramen magnum of the skull was dissected. The brain was carefully removed from the skull, the cerebellum and olfactory bulbs were removed (Picture 2.6), and the midbrain and forebrain were taken out and dissected out, rinsed with phosphate buffer solution, and weighted.



Picture 2.6: Brain dissection

2.9.2. Preparation of phosphate buffer saline

A synthetic blend of inorganic salts known as a "physiological" or balanced salt solution is the basis of all tissue culture mediums (BSS). The salt solution first described by Sydney Ringer is the source of all physiological salt solutions (1885). Tyrode's solution was the first balanced salt solution produced expressly for supporting the metabolism of mammalian cells. Many changes have been made since then to improve buffering salt solutions and prevent calcium precipitation. The function of a salt solution is:

• To keep the pH of the medium within a physiological range.
• To maintain osmotic balance within and outside the cell.
• A carbohydrate, such as glucose, is modified to act as an energy source for cell metabolism. RM7385 is Dulbecco's Phosphate Buffered Saline in tablet

form. One tablet is sufficient to make 100ml of solution Composition: Ingredients Grams/ Liter, Potassium chloride 0.2, Potassium di hydrogen phosphate 0.2, Sodium chloride 8.0, Disodium hydrogen phosphate 1.15. Directions: Dissolve 10 tablets in 1 liter of distilled water and autoclave for 10 min. at 1150C. Store at 15- 30oC away from bright light.

2.9.3. Steps of preparing brain's samples

• homogenization of Brain: residual blood was removed by washing with pre-cooling PBS buffer (pH=7.4).

•Brain was homogenized after weighing, then it was homogenized in PBS (pH=7.4) with a homogenizer on ice.

•Defrosting at 2-8C or freezing at -20C.

•After defrosting, the homogenates were then centrifuged at 2000-3000 RPM for 20min.

2.9.4. Preparation of Tissues for Study

1- Fixation

2- dehydration

3- **clearing**

4- Embedding

5- Sectioning

6- Staining

7- Mounting

The basic steps used in tissue preparation for histology are:

1- Fixation

If a permanent section is desired, tissues must be fixed. Fixation is used to:

1- Terminate cell metabolism,

2- Prevent enzymatic degradation of cells and tissues by autolysis (self-digestion).

3- Kill pathogenic microorganisms such as bacteria, fungi, and viruses.

4- Harden the tissue as a result of either cross-linking or denaturing protein molecules

One fixative widely used for light microscopy is formalin, a buffered isotonic solution of 37% formaldehyde. The chemistry of the process involved in fixation of many tissue components is complex and not always well understood. Both formaldehyde and glutaraldehyde, a fixative often used for electron microscopy, react with the amine groups (NH2) of tissue proteins, preventing their degradation. Glutaraldehyde reinforces this fixing activity by being a dialdehyde capable also of cross linking proteins.

2. Embedding & Sectioning

Tissues are embedded in a solid medium to facilitate sectioning. In order to cut very thin sections, tissues must be infiltrated after fixation with embedding material that imparts a rigid consistency to the tissue. Embedding materials include paraffin and plastic resins; paraffin is used routinely for light microscopy, resins for both light and electron microscopy

Paraffin embedding, is preceded by two other main steps: dehydration and clearing. In dehydration, water is extracted from the fixed tissues by successive transfer through a graded series of ethanol and water mixtures, usually from 70% to 100% ethanol. The ethanol is then replaced by an organic solvent miscible with both alcohol and the embedding medium.

Staining

Most cells and extracellular material are completely colorless, and to be studied microscopically sections must typically be stained (dyed). Methods of staining have been devised that not only make the various tissue components conspicuous but also permit distinctions to be made between them..Dyes stain tissue components more or less selectively, with many behaving like acidic or basic compounds and forming electrostatic (salt) linkages with ionizable radicals of molecules in tissues. Cell components such as nucleic acids with a net negative charge (anionic) stain more readily with basic dyes and are termed basophilic; cationic components, such as proteins with many ionized amino groups, have affinity for acidic dyes and are termed acidophilic Examples of basic dyes are toluidine blue, alcian blue, and methylene blue. Hematoxylin behaves like a basic dye, staining basophilic tissue components. The main tissue components that ionize and react with basic dyes do so because of acidsin their composition (DNA, RNA, and glycosaminoglycans). Acid dyes (eg, eosin, orange G, and acid fuchsin) stain the acidophilic components of tissues such as mitochondria, secretory granules, and collagen. Of all staining methods, the simple combination of hematoxylin and eosin (H&E) is used most commonly. Hematoxylin produces a dark blue or purple color, staining DNA in the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrixof

cartilage). In contrast, eosin stains other cytoplasmic components and collagen pink.

2.10. Biochemical Assessments

2.10.1. Assessments of MDA using ELISA kit:

Malondialdehyde (MDA) is an accepted marker of lipid oxidative damage. Malondialdehye was produced when highly reactive oxygen metabolites, particularly hydroxyl radicals, act on unsaturated fatty acids of phospholipids components of membranes(Deokar P., Jagtap A.,2019) MDA was measured by enzyme linked immunosorbent assay (ELISA).

2. 10. 1. 1. Principle

Kit is an Enzyme Linked Immunosorbent Assay (ELISA). RAT MDA antibody had been pre-coated on the plate. MDA from the sample was put to the wells, where it linked to Abs. After that, the biotinylated Rat MDA Antibody was added to the sample, where it bound to MDA. The biotinylated MDA antibody was then bound by "Streptavidin-HRP". Unbound "Streptavidin-HRP" was washed away during a washing step after incubation. After then, the substrate solution was added, and the color developed in accordance to the amount of Rat MDA. The reaction was stopped by the addition of an acidic stop solution and measured the absorbance at 450 nm.Sensitivity:0.023nmol/ml, Standard Curve Range: 0,05-10nmol/ml.

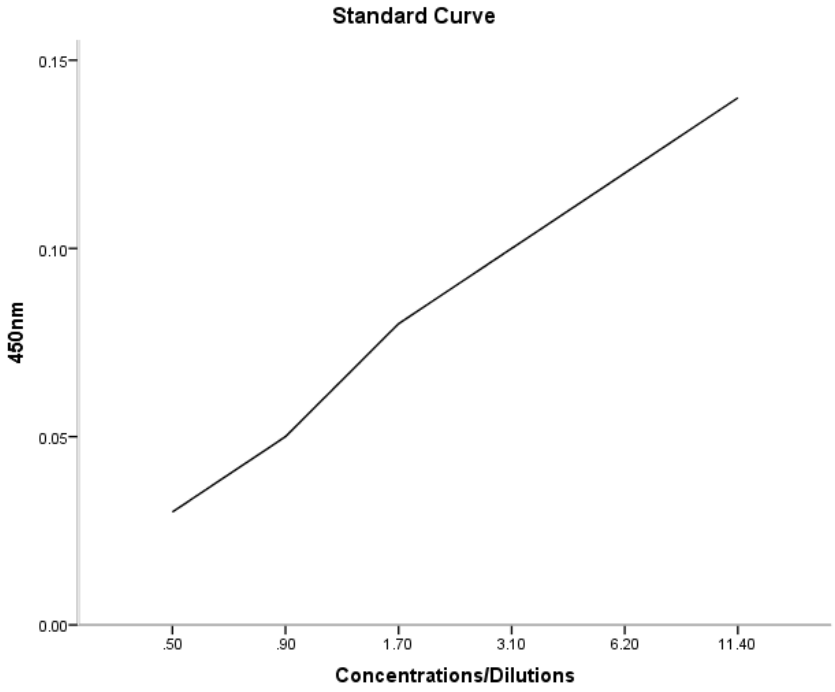


Figure2.1: Standard curve of MDA.

2. 10. 2: Immunohistochemical study

2.10.2.1. Principle

A two-step immunohistochemistry broad spectrum detection agent is 2-Step Plus. In place of the secondary antibody and tertiary antibody used in the conventional approach, it polymerizes monovalent Fab fragments of secondary antibody and enzyme, which can directly increase the binding signal of antibody-antigen. This approach successfully avoids the space-steric barrier brought on by too many polymer molecules while simultaneously maintaining the antibody's capacity to attach to antigens specifically. This kit's advantages over the conventional SP three-step approach include its simplicity, speed, and great sensitivity. In order to prevent endogenous biotin

from coloring the background, the system stops utilising biotin. It can be utilized in IHC, which uses primary antibodies made from rabbit or mouse monoclonal or polyclonal antibodies. Better primary antibody binding detection is made possible by a special polymer auxiliary agent for macromolecular detection. This kit includes a diluent for the concentrated DAB solution to counteract the effects of varying water alkalinity and acidity on the DAB chromogenic agent.Immunohistochemical score for tyrosine hydroxylase :

Histoscore is based on the percentage of cells that fall into each of the four immunohistochemical categories: negative (0), weak (1+), moderate (2+), and stronge(3+) stained membranes. Each case's histoscore, which might vary from 0 to 300, was determined in the manner described below: HistoScore (H-score) is calculated as follows: HistoScore (H-score)=((1 × % weakly stained cells)+(2 × % moderately stained cells)+(3 × % strongly stained cells)) (Kristian et al., 2017)

Picture 2.7: Elisa kit for Immunohistochemical study.

2.11. Analytical Statistics

The SPSS version 20 was used to statistically evaluate the study's findings.One-way ANOVA and the post hoc test are statistical formulas used to determine if differences are statistically significant. Statistical significance was set at 5% thus p value ≤ 0.05 was considered significant.

Chapter Three
Results

3. 1. Weight

In group 1 (control group, untreated and not exposed to rotenone), there were no considerable differences (P value >0.05) in the means of weight on day 15and day 30 as compared with day 0 (Table 3.1 and Figure 3.1). In addition, in group 2 (not treated but exposed to rotenone) the means of weight were considerable decreased (P value < 0.05) on day 30 as compared with day 0 (Table 3. 1 and Figure 3. 1).In group 3 (treated with sinemet and injected rotenone), there were no considerable differences (P value >0.05) in the means of weight on day 15 and day 30 as compared with day 0 (Table 3.1 and Figure 3.1).In group 4 (treated with 500mg/kg S. Officinalis and also exposed to rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone), the means of weight were considerable decreased (P value < 0.05) on day 30 as compared with day 0, while group 4 (treated with 500mg/kg S. Officinalis and exposed to rotenone) and group 5 (treated with 500mg/kg S. Officinalis and also exposed to rotenone) had shown considerable decrease (P value < 0.05) in the means of weight on day 30 as compared with day15 (Table 3.1 and Figure 3.1).

Table 3.1: Comparing in means of weight ± SEM between all groups on days 0, 15, 30

Wt	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Day 0	253.300±15.19693	277.300±14.31662	258.600±16.39856	258.800±9.18699	243.500±18.72407
Day 15	257.700±16.19653	239.600±15.16409	246.400±15.35665	218.200±9.93734	215.400±*18.89156
Day 30	268.300±15.19453	217.9±* Ω 12.29695	234.700±15.02894	193.1± * Ω 8.86999	218.7±* 18.07444

* = significantly decreased (p value <0.05) as compared with day 0.

Ω = significantly decreased (p value <0.05) as compared with day 15.

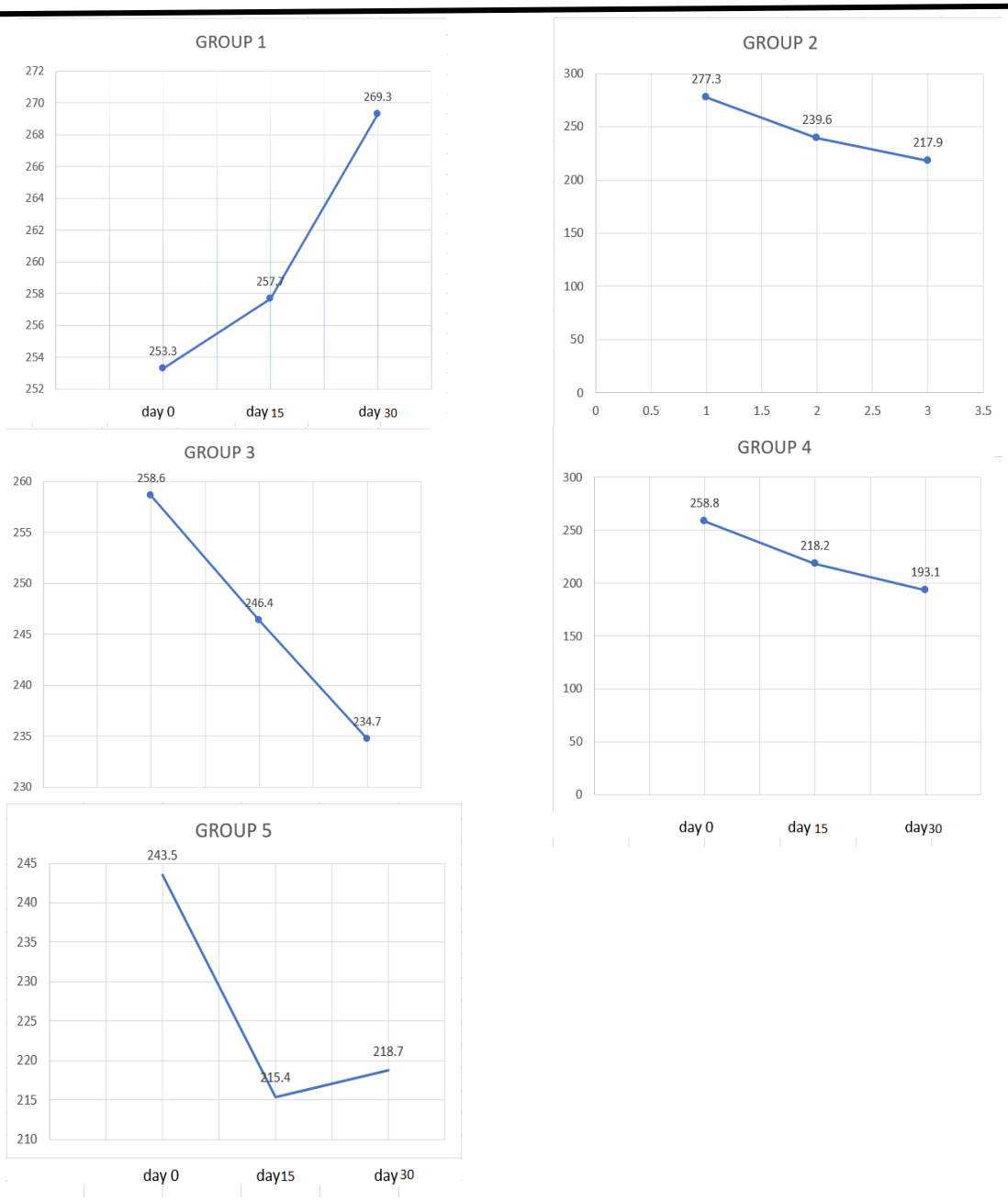


Figure 3.1: The means of body weights on days 0, 15, 30 for all groups

Group 1 (control group, untreated and unexposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone) . No. of rat = 10 rats for each group.

3. 2. The Rotarod Test

3. 2. 1. Number of Rotations

The number of rotations significantly decreased (P value <0.05) in group 2, group 3, group 4 as compared with group 1, while, the number of rotations significantly increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2, furthermore the number of rotations insignificantly decreased (P value >0.05) in group 5 as compared with group 1. (Table 3.2 and Figure 3.2).

Table 3. 2: A comparing mean differences of number of rotations between the groups

no. of rotations	G. 1	G. 2	G. 3	G. 4	G. 5
G. 1	X	9.300*	5.548*	5.718*	2.946
G. 2	-9.300*	X	-3.746*	-3.578*	-6.347*
G. 3	-5.548*	3.746*	X	0.169	-2.603
G. 4	-5.718*	3.578*	-0.165	X	-2.769
G. 5	-2.946	6.347*	2.598	2.769	X

* The mean difference is significant at the 0.05 level.

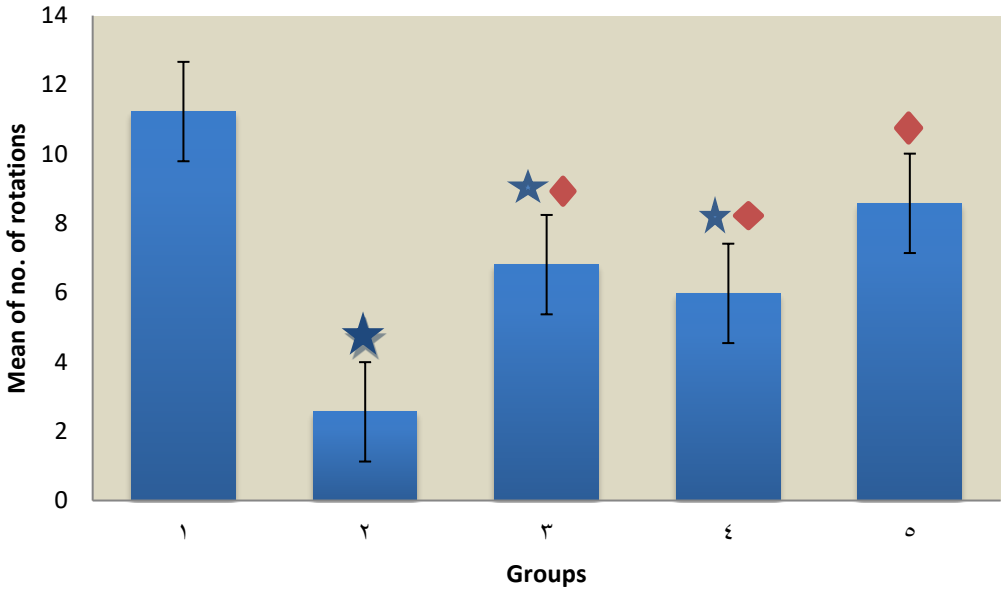


Figure 3.2: Means of rotations no. ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone),group 5 (treated with 500mg/kg S. Officinalis and injected rotenone). no. of rat = 10 rats for each group.

★= significantly decreased (p value <0.05) when compared with group 1.
◆= significantly increased (p value <0.05) when compared with group 2.

3. 2. 2. Rotations Distance

Rotations distance considerably decreased (P value <0.05) in group 2, group 3, group 4 and group 5 as compared with group 1. furthermore, the rotations distance considerably increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2 (Table 3.3 and Figure 3.3).

Table 3.3: Comparing the mean differences of rotations distance (cm) between the groups.

Rotation distance	Group1	Group2	Group3	Group4	Group5
Group1	X	121.91*	81.82*	93.14*	32.54*
Group2	-121.91*	X	-40.09*	-28.77*	-89.37*
Group3	-81.82*	40.09*	X	11.32*	-49.28*
Group4	-93.14*	28.77*	-11.32*	X	-60.6*
Group5	-32.54*	89.37*	49.28*	60.6*	X

* The mean difference is significant at the 0.05 level.

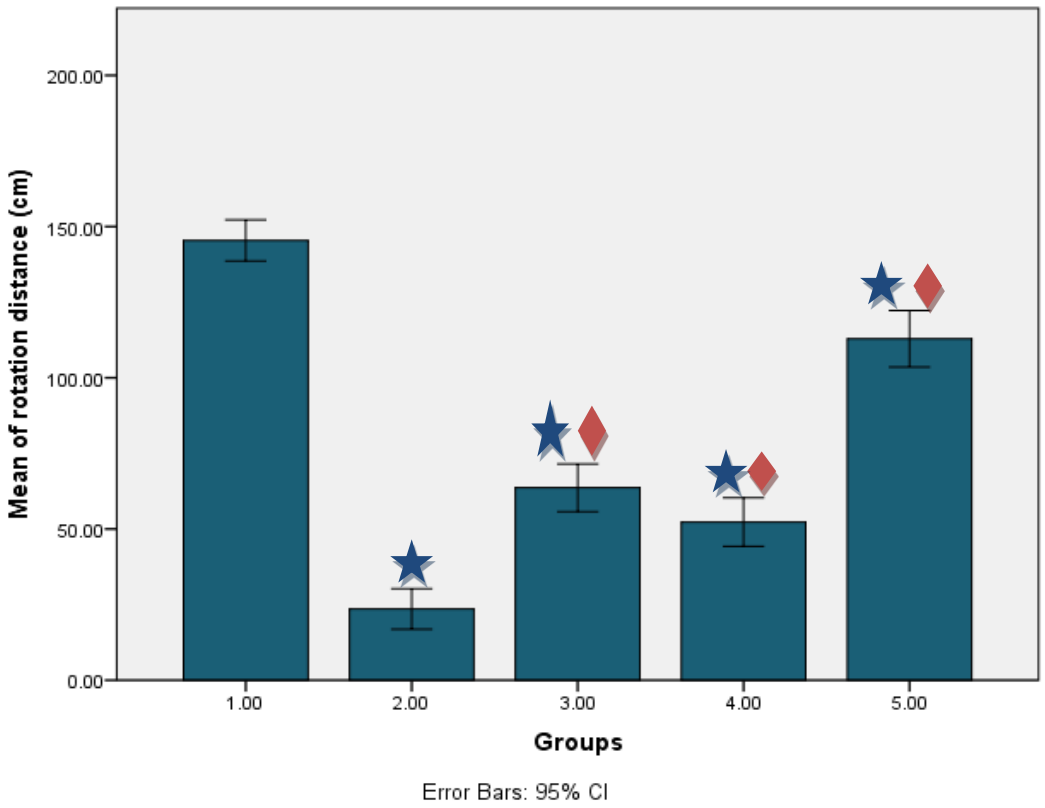


Figure 3.3: Means of rotations distance (cm) ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly decreased (p value <0.05) when compared with group 1.
◆ = significantly increased (p value <0.05) when compared with group 2.

3. 2. 3. Time of Rotations

The time of rotations considerably decreased (P value <0.05) in group 2, group 3, group 4 and group 5 when compared with group 1. Furthermore, the time of rotations considerably increased (P value <0.05) in group 3, group 4, group 5 when compared with group 2 (Table 3.4 and Figure 3.4).

Table 3.4: Comparing the means differences of rotation time between the groups

Time of rotation	Group1	Group2	Group3	Group4	Group5
Group1	X	25.2*	14.8*	17.5*	7.8*
Group2	-25.2*	X	-10.4*	-7.7*	-17.4*
Group3	-14.8*	10.4*	X	2.7	-7.0
Group4	-17.5*	7.7*	-2.7	X	-9.7*
Group5	-7.8*	17.4*	7.0	9.7*	X

* The mean difference is significant at the 0.05 level.

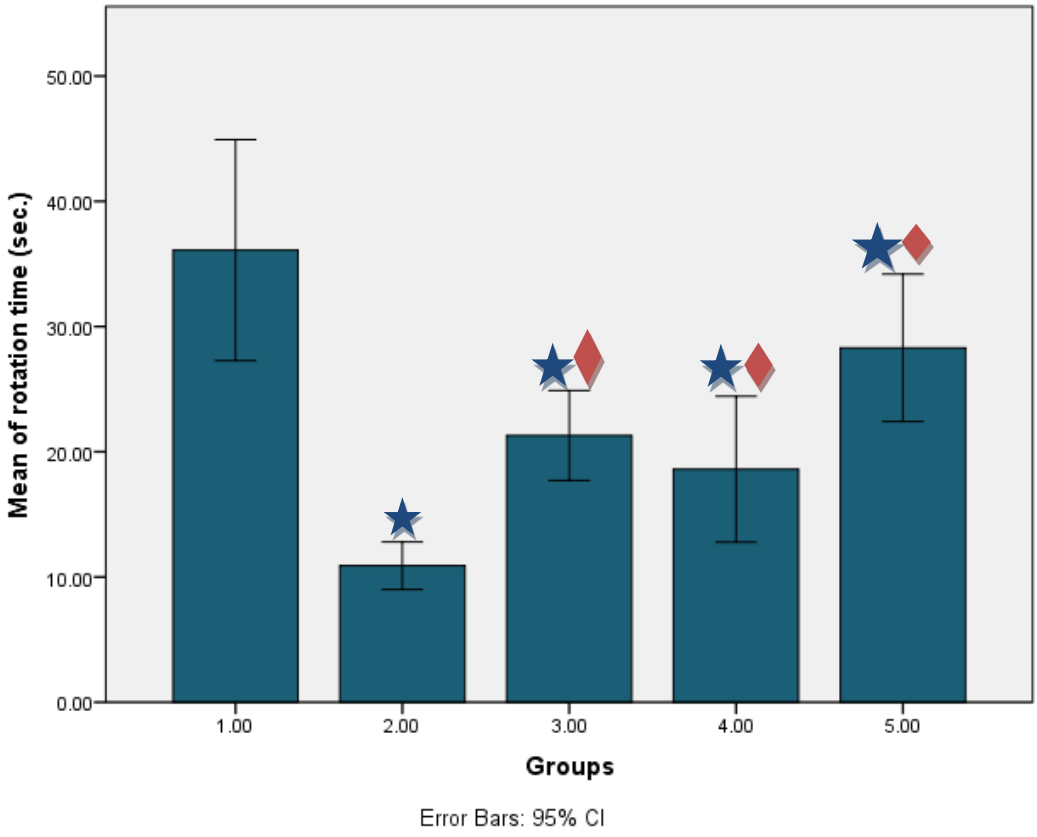


Figure 3.4: Means of rotations time (sec) ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly decreased (P value <0.05) when compared with group 1.
♦ = significantly increased (P value <0.05) when compared with group 2.

3. 3. Open Field Tests

3. 3. 1. Crossing (traveled distance)

The traveled distance by the rats considerably decreased (P value <0.05) in group 2, group 3, group 4 when compared with group 1 while considerably increased (P value <0.05) in group 3, group 4, group 5 when compared with group 2 (Table 3.5 and Figure 3.5). Moreover, the no. of squares which are crossed by rats considerably increased (P value <0.05) in group 5 when compared with group 4 (Table 3.5 and Figure 3.5).

Table 3.5: Comparing the mean differences of crossing (no. Of squares) between different groups

no. of crossing	Group1	Group2	Group3	Group4	Group5
Group1	X	171.5*	62.5*	74.5*	36.1
Group2	-171.5*	X	-109.4*	-97.0*	-135.4*
Group3	-62.1*	109.4*	X	12.4	-26.0
Group4	-74.5	97.0*	-12.4	X	-38.4*
Group5	-36.1	135.4*	26.0	38.4*	X

* . The mean difference is significant at the 0.05 level.

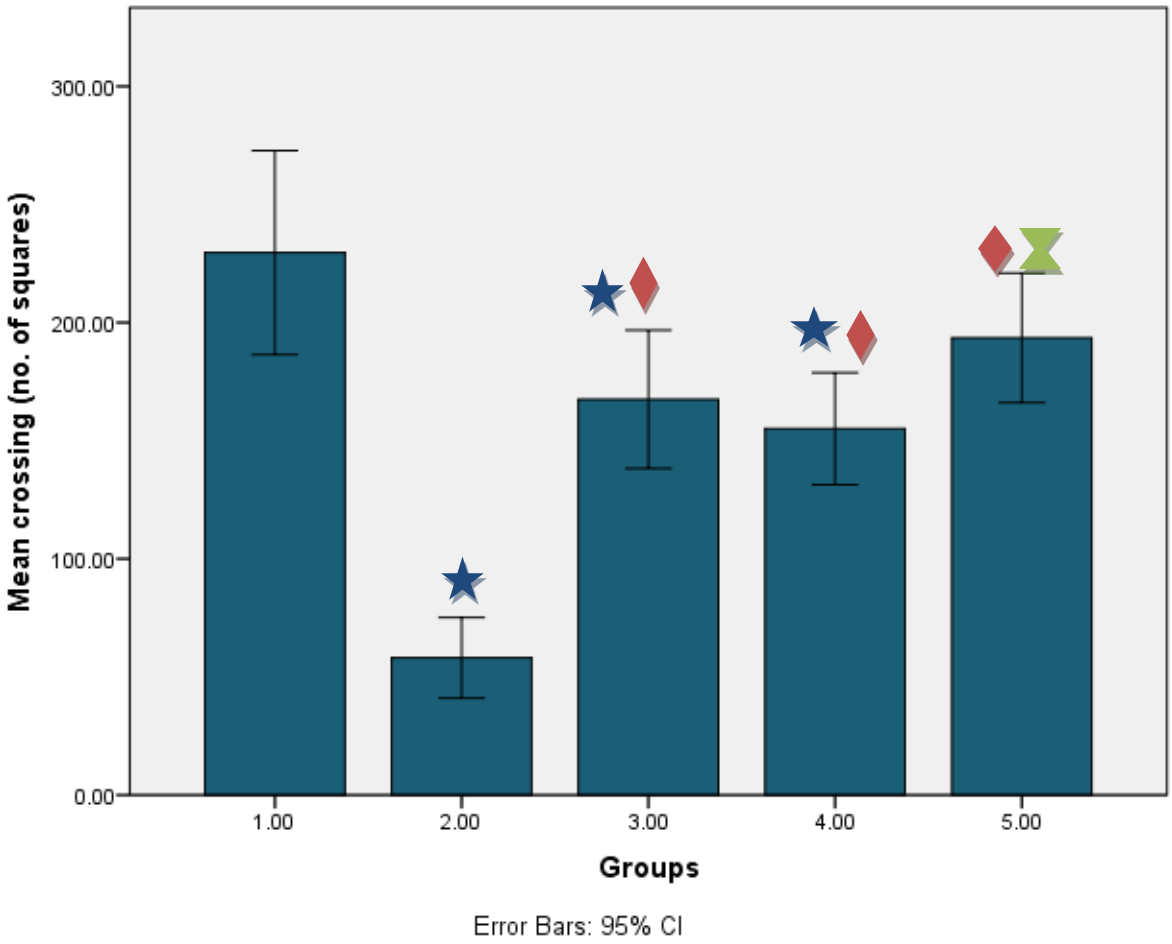


Figure 3.5: Means of crossing (no. of squares) ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.
★ = significantly decreased (P value <0.05) when compared with group 1.
♦ = significantly increased (P value <0.05) when compared with group 2.
⧗ = significantly increased (P value <0.05) when compared with group 4.

3. 3. 2. Risk Factors (no. of visits to center area)

The no. of visits to center area (risk factor) considerably decreased (p value <0.05) in group 2, group 3 and group 4 as compared with group 1 while considerably increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2.Furthermore it insignificantly decreased in group5 when compared with group 1 (Table 3.6 and Figure 3.6).

Table 3.6: Comparing the mean differences of no. of visits to center area (risk factor) between the groups

RF	Group1	Group2	Group3	Group4	Group5
Group1	X	4.6*	1.7*	-1.6*	1.1
Group2	-4.6*	X	-2.9*	-3.0*	-3.5*
Group3	-1.7*	2.9*	X	-0.1	-0.6
Group4	-1.6*	3.0*	0.1	X	-0.5
Group5	-1.1	3.5*	0.6	0.5	X

* The mean difference is significant at the 0.05 level.

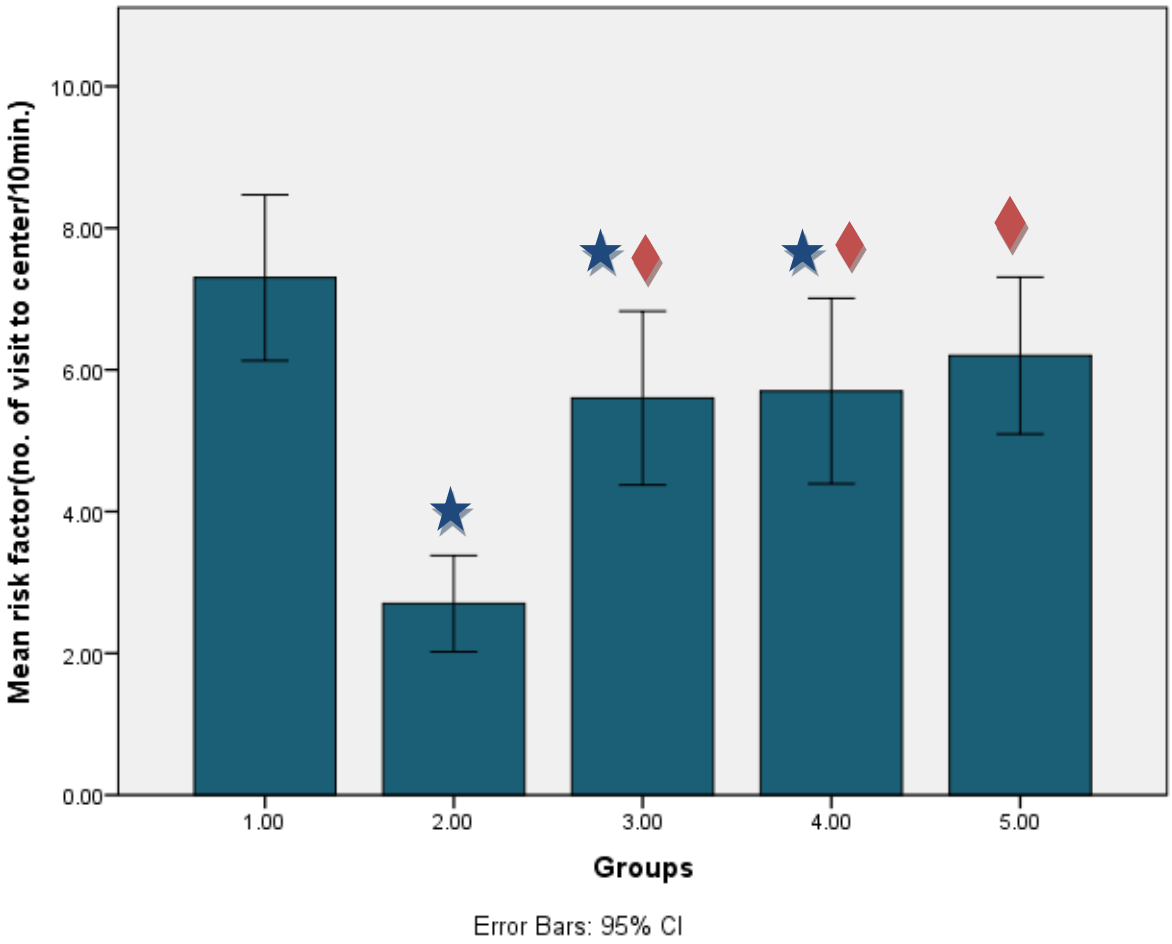


Figure 3.6: Means of no. of visits to center area (risk factor) ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly decreased (P value <0.05) as compared with group 1.
♦ = significantly increased (P value <0.05) as compared with group 2.

3. 3. 3. Number of rearing

No. of rearing considerably decreased (P value <0.05) in group 2, group 3 and group 4 as compared with group 1 while considerably increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2. Furthermore it insignificantly decreased in group5 when compared with group 1 (Table 3.7 and Figure 3.7).

Table 3.7: Comparing the mean differences of no. of rearing between the groups

Rearing	Group1	Group2	Group3	Group4	Group5
Group1	X	17.9*	7.7*	8.6*	3.0
Group2	-17.9*	X	-10.2*	-9.3*	-14.9*
Group3	-7.7*	10.2*	X	0.9	-4.7*
Group4	-8.6*	9.3*	-0.9	X	-5.6*
Group5	-3.0	14.9*	4.7*	5.6*	X

* The mean difference is significant at the 0.05 level.

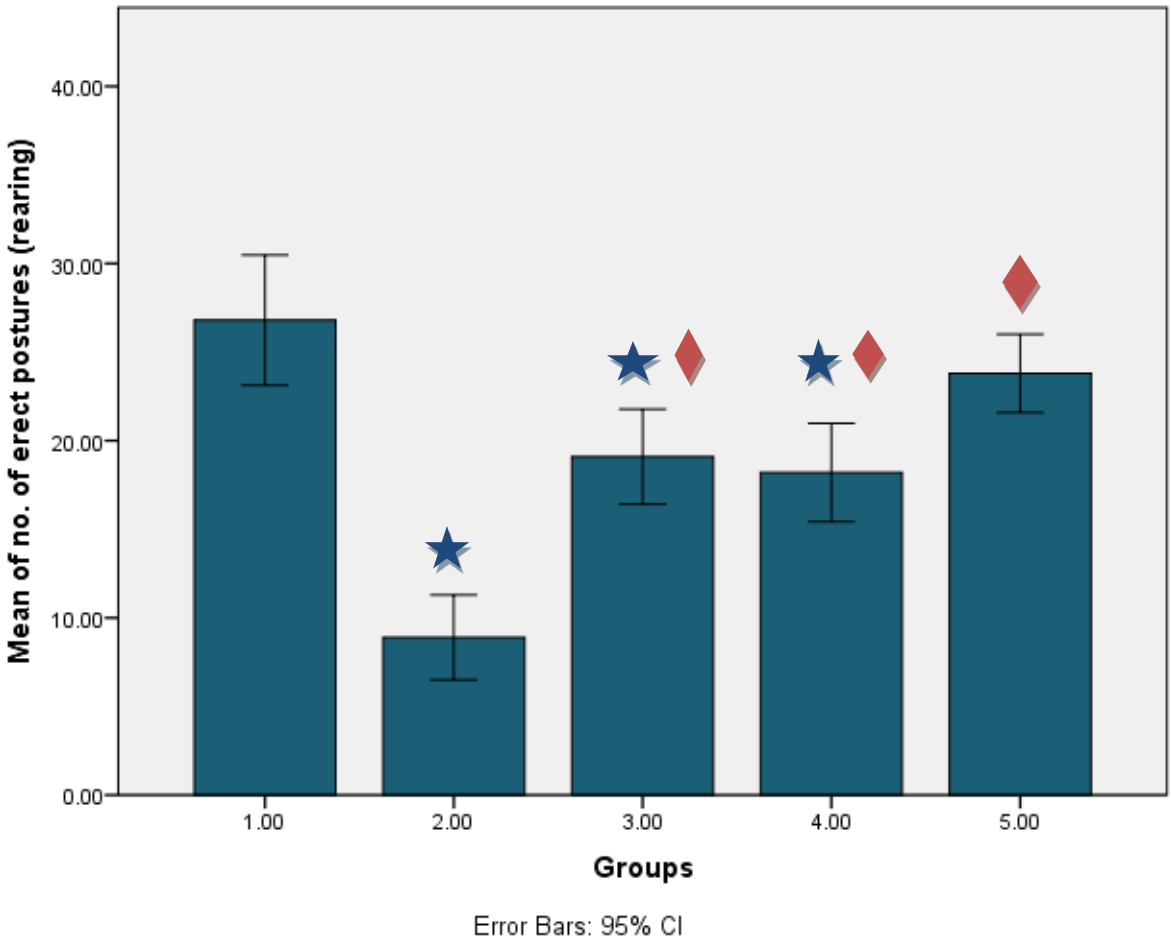


Figure 3.7: Means of no. of rearing ± SEM in all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly decreased (P value <0.05) when compared with group 1.
◆ = significantly increased (P value <0.05) when compared with group 2.

3. 3. 4. Grooming Time

Grooming time considerably decreased (P value <0.05) in group 2, group 3, group 4and group 5 as compared with group 1 while considerably increased (P value <0.05) in group 3, group 4, group 5 when compared with group 2 (Table 3.8 and Figure 3.8).

Table 3.8: Comparing the means differences of grooming time between the groups

Grooming time	Group1	Group2	Group3	Group4	Group5
Group1	X	21.31*	16.63*	11.91*	6.0*
Group2	-21.31*	X	-10.68*	-9.4*	-15.31*
Group3	-10.63*	10.68*	X	1.28	-4.63
Group4	-11.91*	9.4*	-1.28	X	-5.91*
Group5	-6.0*	15.31*	4.63	5.91*	X

* The mean difference is significant at the 0.05 level.

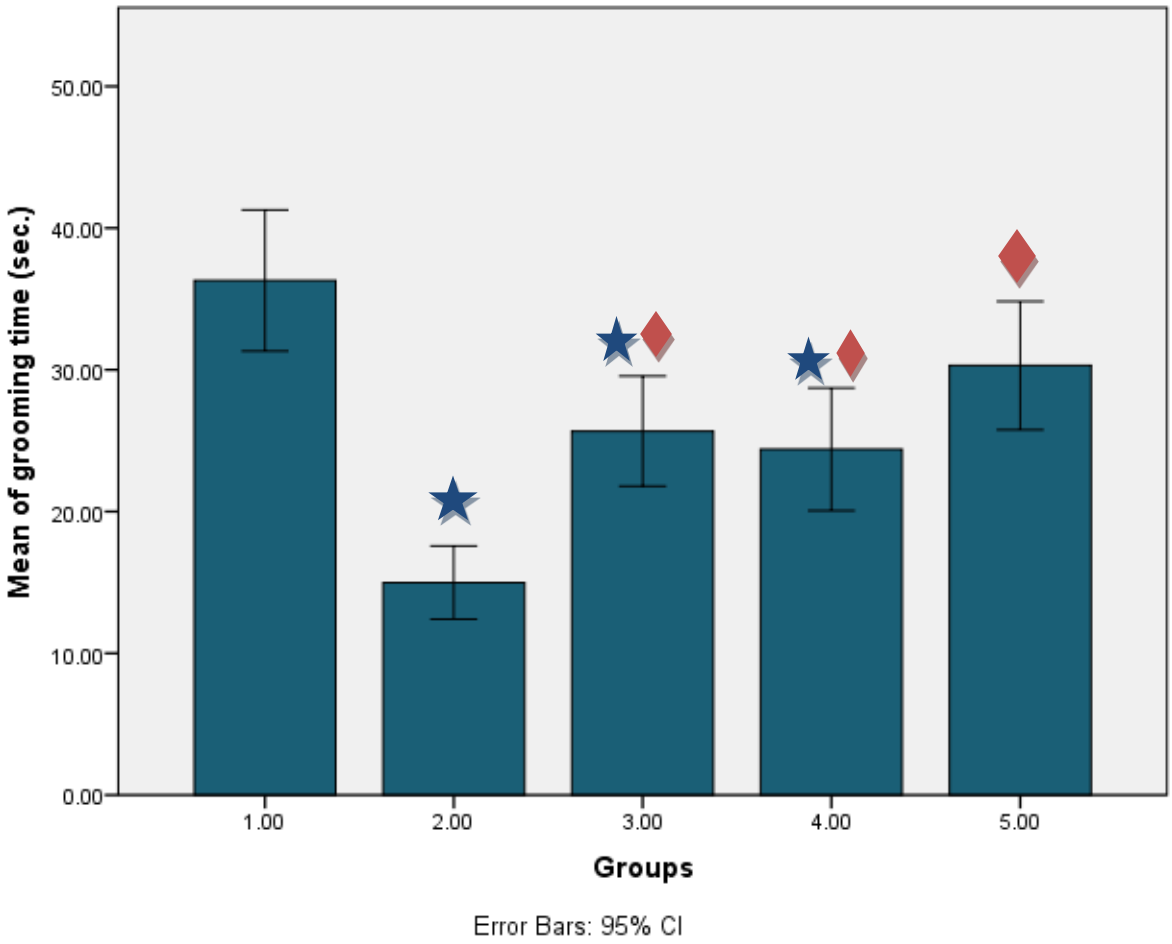


Figure 3.8: Means of grooming time (sec) ± SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly decreased (P value <0.05) when compared with group 1.
♦ = significantly increased (P value <0.05) when compared with group 2.

3. 4. the latency of falling time test (Force Gripping test)

In force griping test, the falling time latency considerably decreased (P value <0.05) in group 2, group 3 and group 4 and group 5 when compared with group 1 while considerably increased (P value <0.05) in group 3, group 4 and group 5 when compared with group 2 (Table 3.9 and Figure 3.9).

Table 3.9: Comparing the mean differences of falling time latency in force griping test between the groups

Falling time	Group1	Group2	Group3	Group4	Group5
Group1	X	16.337*+	7.175*	8.505*	3.655*
Group2	-16.337*	X	-9.162*	-7.832*	-12.682*
Group3	-7.175*	9.162*	X	1.33	-3.52
Group4	-8.505*	7.832*	-1.33	X	-4.85*
Group5	-3.655	12.682*	3.52	4.85*	X

* The mean difference is significant at the 0.05 level.

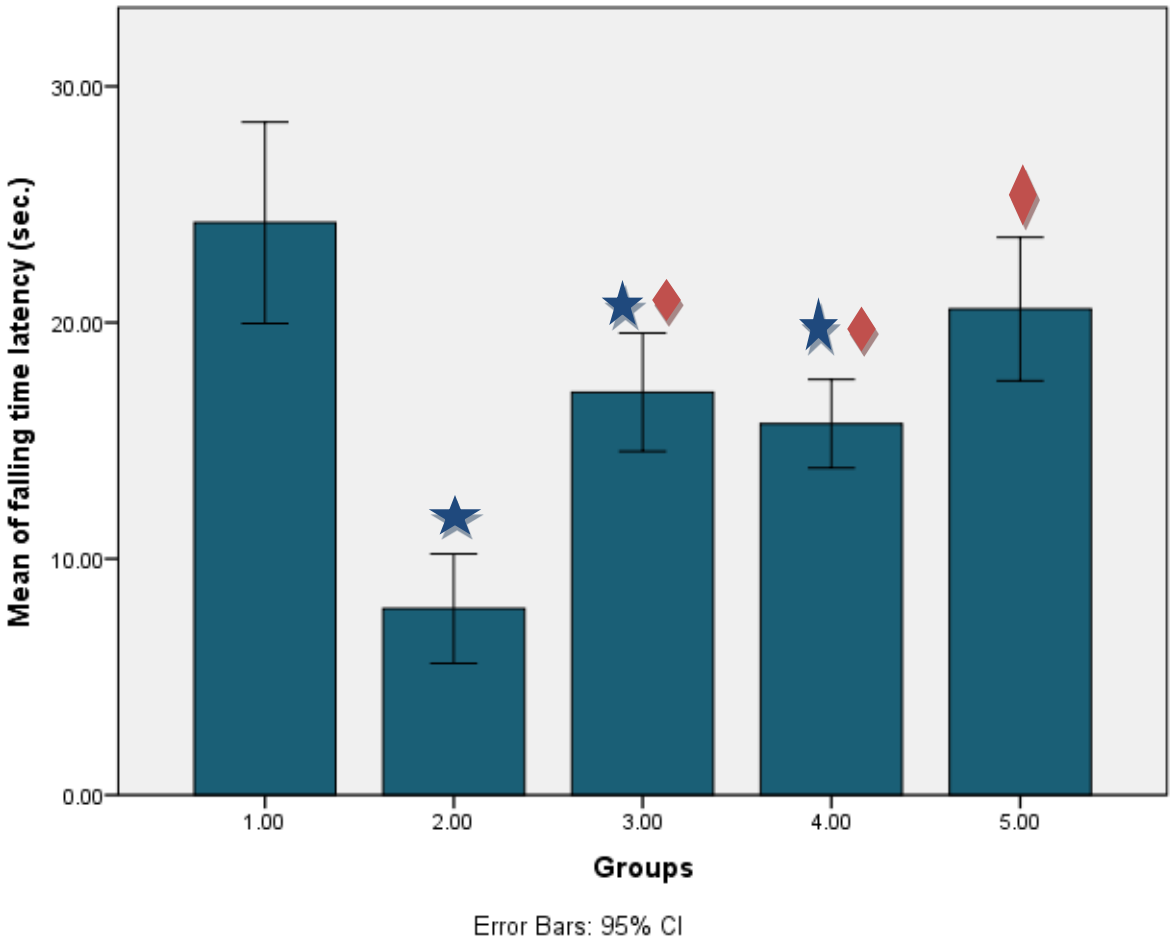


Figure 3.9:Means of falling time latency in force griping test (sec) ± SEM in all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★= significantly decreased (P value <0.05) when compared with group 1.
♦ = significantly increased (P value <0.05) when compared with group 2.

3. 5.Biochemical study

3.5.1. Malondialdehyde levels

Malondialdehyde (MDA) levels considerably increased (P value <0.05) in group 2 and group 3 as compared with group 1 while considerably decreased (P value <0.05) in group 4 and group 5 as compared with group 2 and group 3, Furthermore group 4 and group 5 insignificantly decreased as compared with group 1 (Table 3.10 and Figure 3.10).

Table 3.10: Comparing the mean differences of MDA levels between different groups

MDA	Group1	Group2	Group3	Group4	Group5
Group1	X	3.43*	-1.963*	0.945	0.339
Group2	3.432*	X	1.469	4.377*	3.771*
Group3	1.963*	-1.469	X	2.908*	2.302*
Group4	-0.945	-4.377*	-2.908*	X	-0.606
Group5	-0.339	-3.771*	-2.302*	0.606	X

* The mean difference is significant at the 0.05 level.

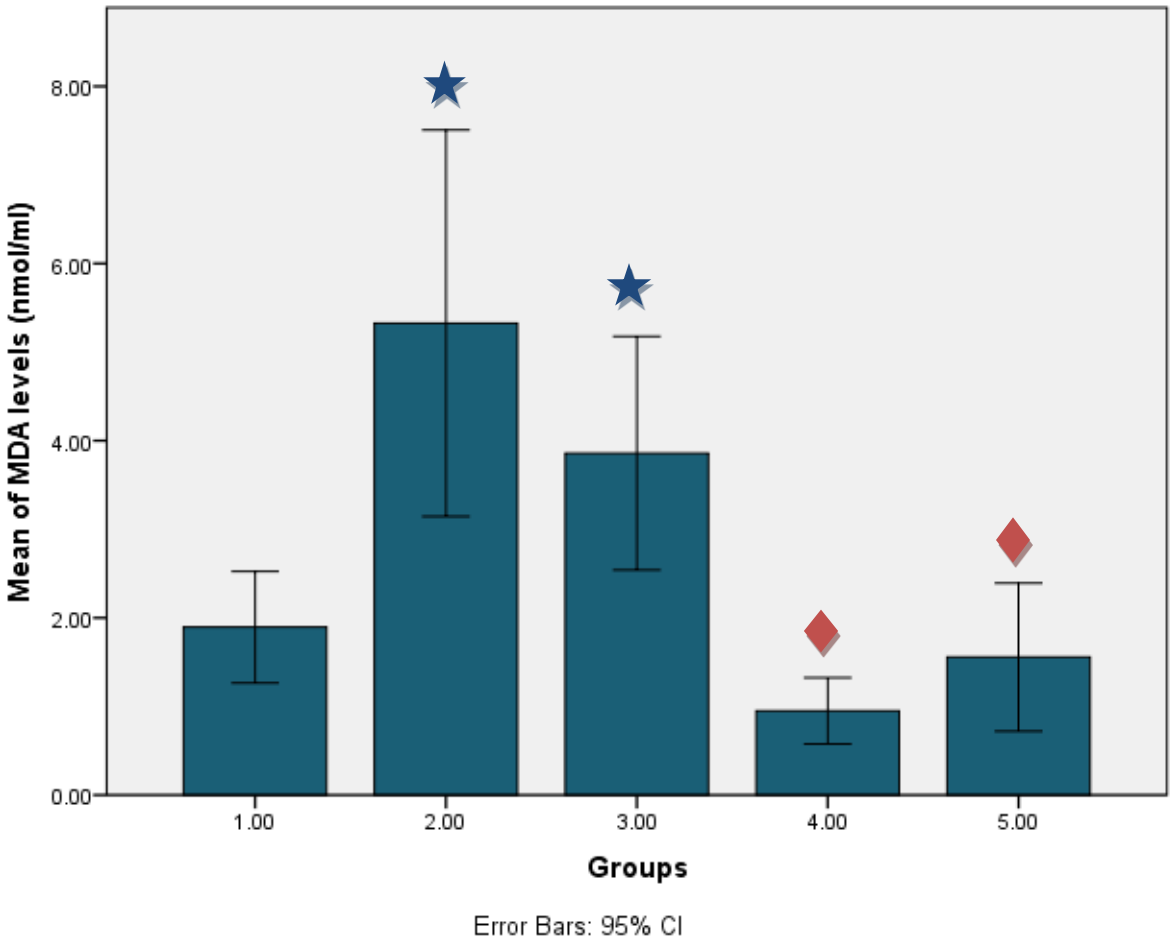


Figure 3.10: Means of MDA levels (nmol/ml) ± SEM of all groups

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). no. of rat = 10 rats for each group.

★ = significantly increased (p value < 0.05) when compared with group 1.
◆ = significantly decreased (p value < 0.05) when compared with groups 2 and3.

3.6. Immunohistochemical study (IHC)

3.6.1. Number of neuron cells with positive tyrosine hydroxylase

Considerably decreased (P value <0.05) in group 2, group3, group4 and group 5when compared with group 1 while considerably increased (P value <0.05) in group 3, group 4 and group 5 when compared with group 2 (Table 3.11 and Figure 3.11).

Table 3.11: Comparing mean differences of number Of neurons with +ve stain between different groups

No. of neurons	Group1	Group2	Group3	Group4	Group5
Group1	X	58.1*	38.7*	47.2*	32.1*
Group2	-58.1*	X	-19.4*	-10.9*	-26.0*
Group3	-38.7*	19.4*	X	8.5*	-6.6
Group4	-47.2*	10.9*	-8.5*	X	-15.1*
Group5	-32.1*	26*	6.6	15.1*	X

* The mean difference is significant at the 0.05 level.

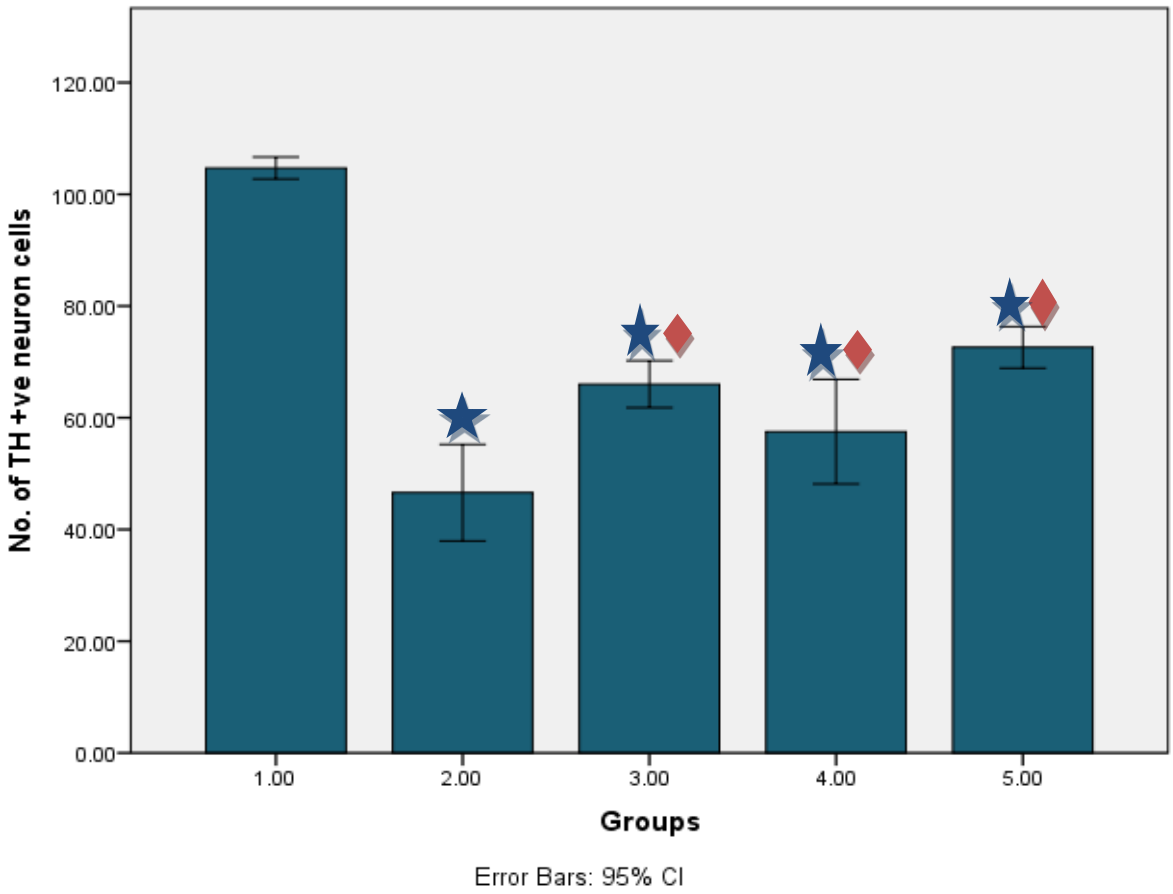


Figure 3.11: Means of no. Of neurons with +ve stain ±SEM of all groups.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and injected rotenone). no. of rat = 10 rats for each group.

= significantly decreased (p value < 0.05) when compared with group1.
= significantly increased (p value < 0.05) when compared with group2.

In order to determine the Histoscore of various groups at magnification (40x), IHC was carried out in this study on formalin-fixed, paraffin-embedded tissue samples using an SNCA poly clonal antibody and a 2-step plus poly-HRP anti-rabbit/mouse IgG detection system (with DAB solution) Based on four categories of immunohistochemistry that are reported as a percentage of cells, the HistoScore is called Intensity of Neuron with Positive Stain (BAD Stain): negative (0), weak (1+), moderate (2+), and strongly (3+) stained membranes.

In each case, a histo Score with a possibility range of 0–300 was counted as follows: Histo Score (H-score) = ((1×% weakly stained cells) + (2×% moderately stained cells) + (3×% strongly stained cells))

Table 3.12: Histoscore (intensity of neurons with positive stain) as %

Groups	Histoscore
G1	351.65
G2	198.84
G3	250.756
G4	235.65
G5	255.0285

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and injected rotenone). no. of rat = 10 rats for each group.

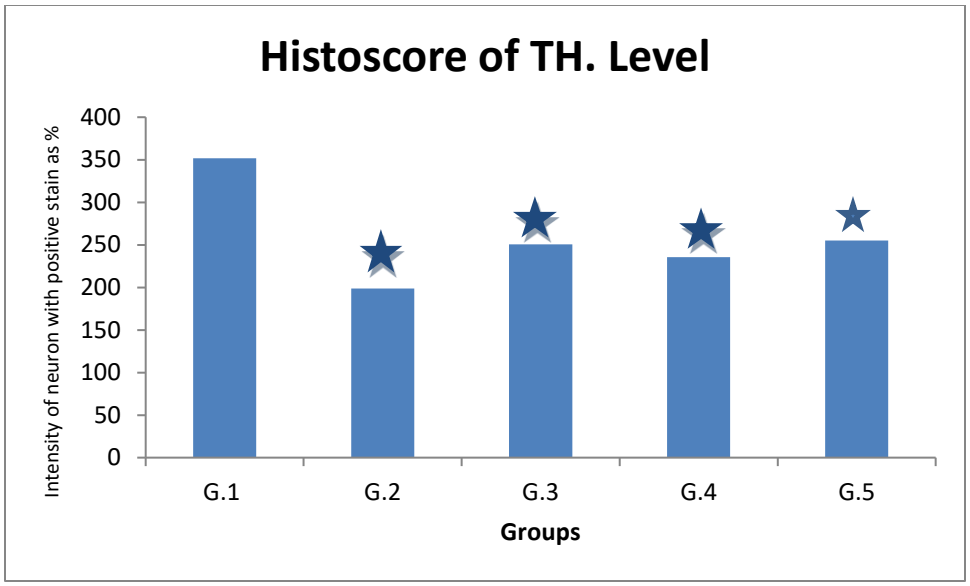


Figure 3.12: No. Of TH +ve neurons as %

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and injected rotenone). no. of rat = 10 rats for each group.

★= significantly decreased (p value < 0.05) when compared with group1.

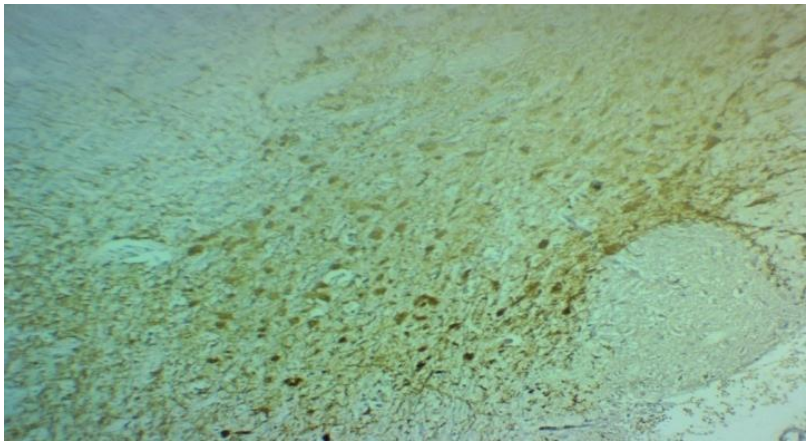


Figure 3.13: Histological section of SNPs in G1 a group treated with Distal water show positive stain in neurons and the fibers (HIC with BAD stain 40x)

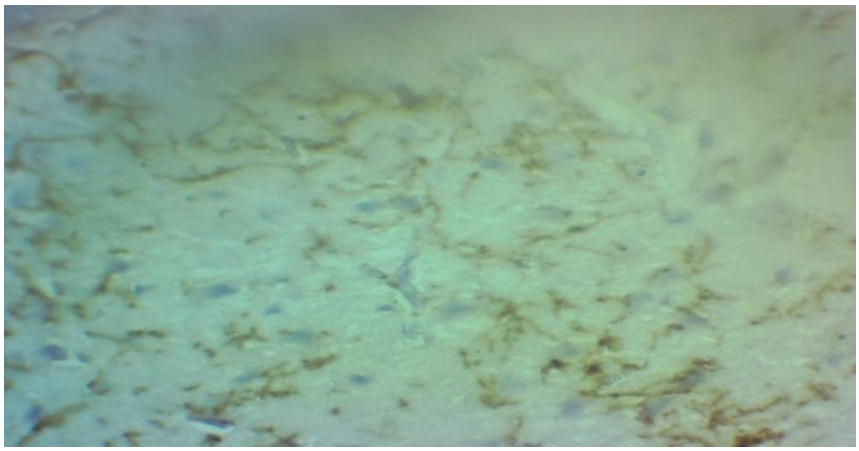


Figure 3.14: Histological section of SNPs in G2 a group treated with Rotenone show negative stain in neurons and only the fibers stained (HIC with BAD stain 40x) .

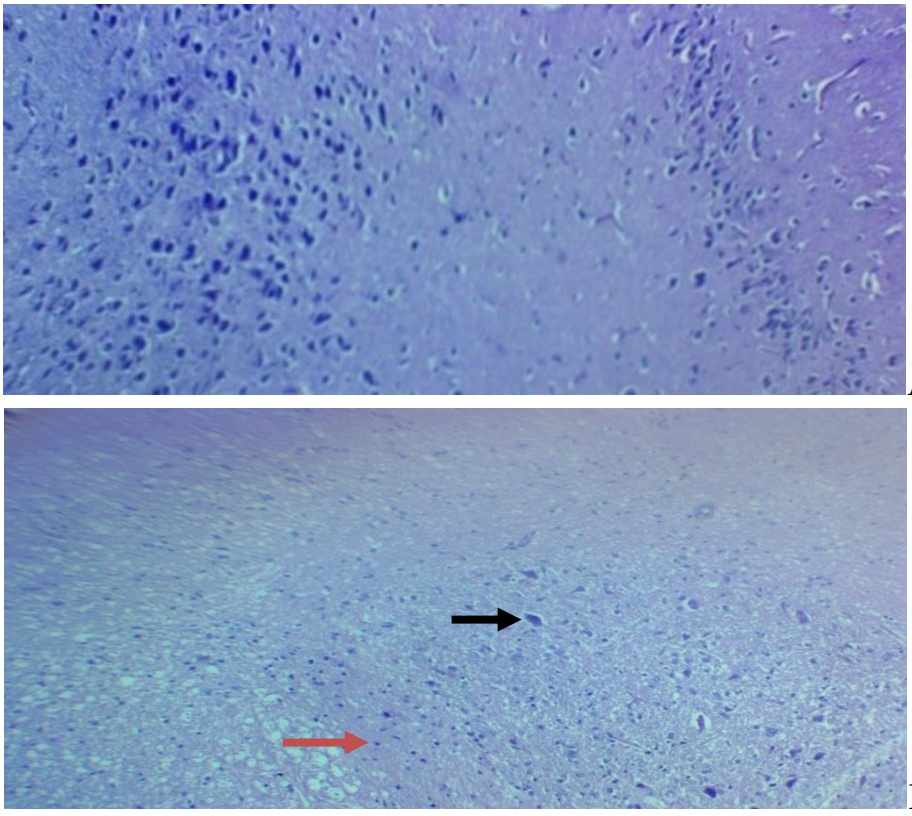


Figure 3.15:Histological section of mid brain showing the neurons density and size .In control group (A) and Rotenone group (B) black rows refers to neurons and red row refers to neuroglia most of them astrocyte (H&E 10X)

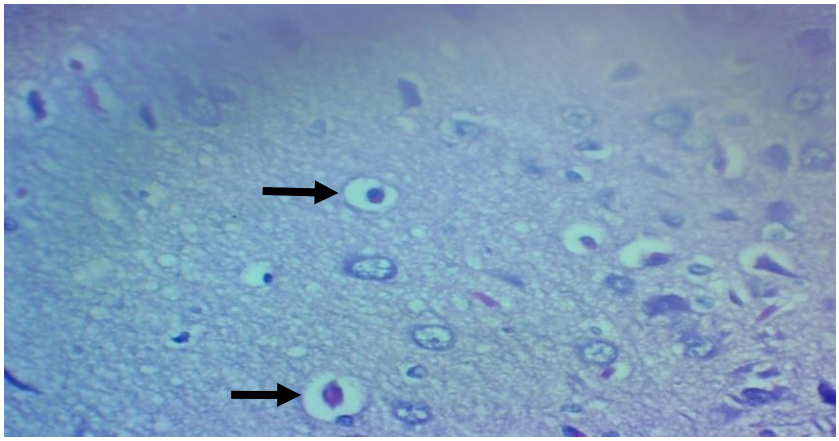


Figure 3.16: Lowy bodies in the SNPs in the rotenone – induced rats. Arrow marks show the Lowy bodies in the substantia nigra pars compacta neurons of G2 animals exposed to rotenone IP to induce Parkinson's disease.

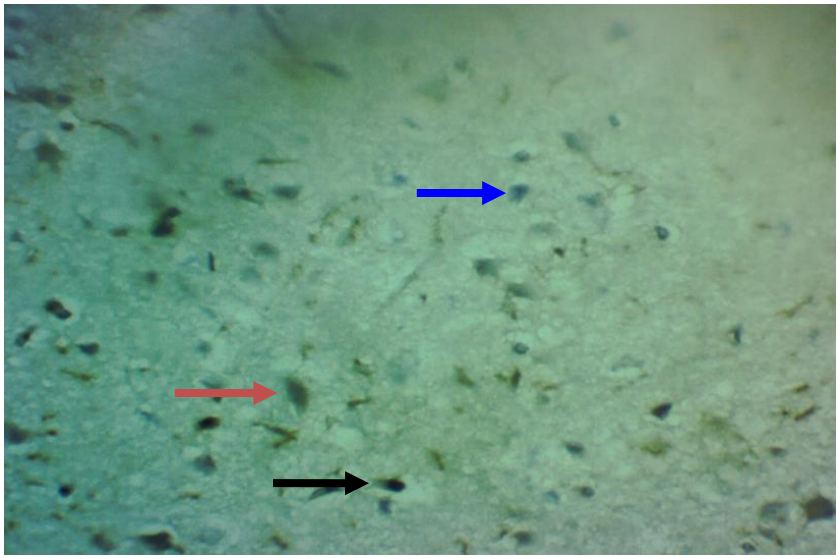


Figure 3.17: Histological section of substantia nigra of mid brain showing the various acceptance in group 3 (R + O) black rows refers to neurons which has strong stain red rows refers to meddle stain while blue refers to weak stain (BAD and 40X)

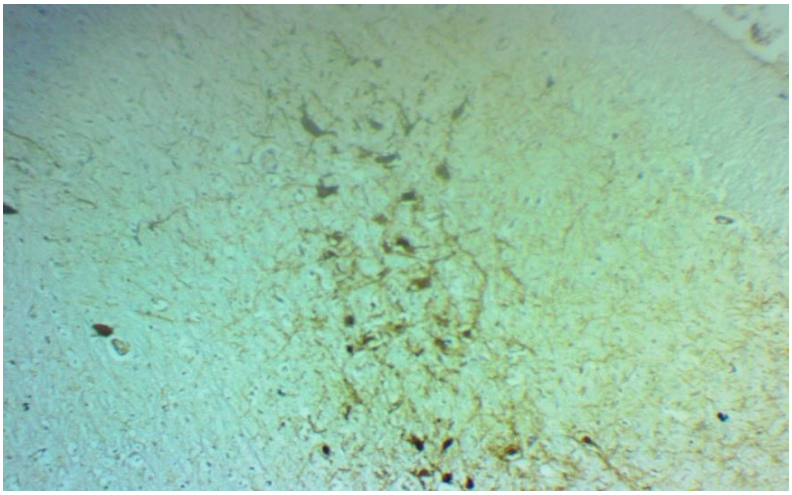


Figure3.18: Histological section of SNPs in G3 a group injected with rotenone treated with sinemet show positive stain in neurons and the fibers (HIC with BAD stain 10x)

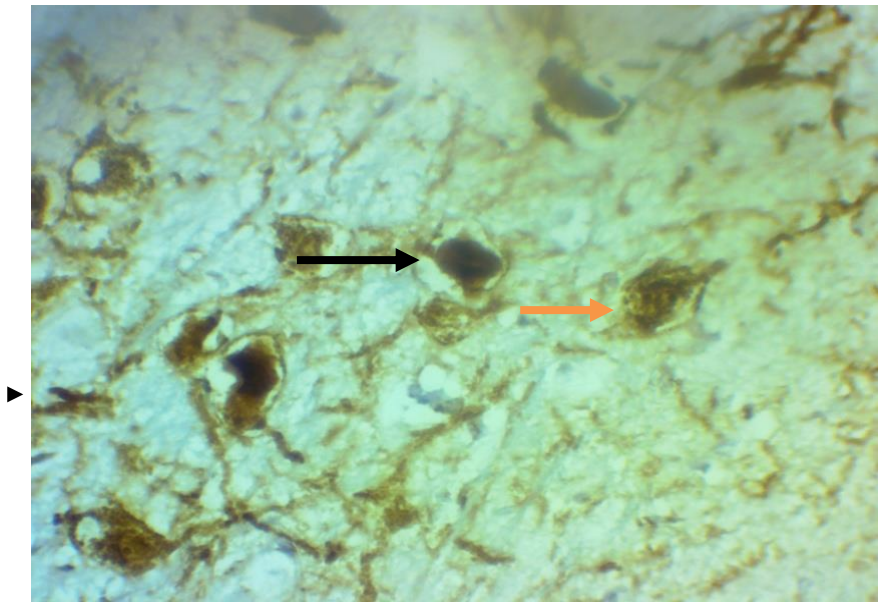


Figure 3.19: Histological section of SNPs in G4 a group injected with rotenone and treated with Salvia officinalis show strongly stained (Black Arrow) and weak stained (Orange Arrow) (HIC with BAD stain 40x).

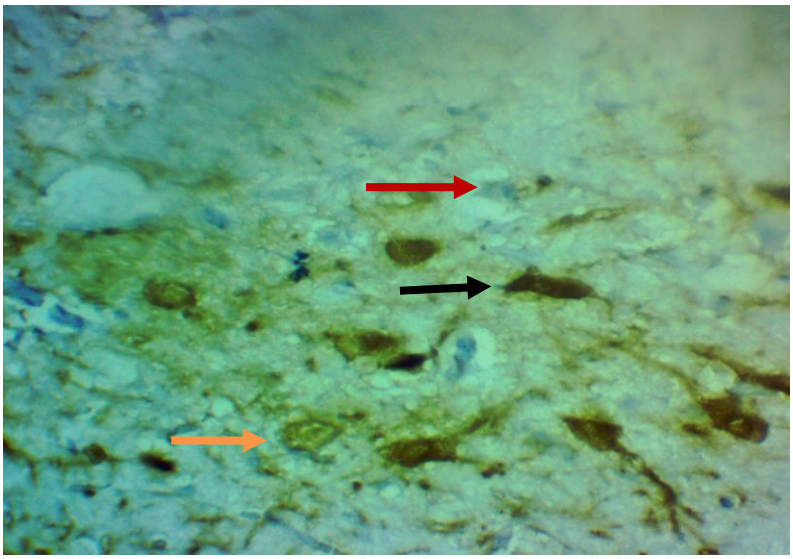


Figure 3.20: Histological section of SNPs in G5 a group injected with rotenone treated with Salvia officinalis and sinemet show strongly stained (Black Arrow) and weak stained (Orange Arrow) and red row negative stain (HIC with BAD stain 40x)

Chapter Four
Discussion

4.1.Introductory Remarks

Although the exact mechanism behind the pathophysiology of Parkinson disease is still unknown, oxidative stress and neuro-inflammation have been closely linked to the death of dopaminergic neurons in the SNc, which leads to striatal DA reduction (Heneka, Kummer and Latz 2014).In the present study, repeated systemic administration of rotenone (2.5 mg/kg doses, IP) in rats produced increased midbrain lipid peroxidation and impaired anti-oxidant status, accompanied by histological changes. Rotenone still remains a preferred model that consistently simulates the neuropathological features of PD because of its ability to reproduce the progressive nature of PD with characteristic slowness of cell death and motor impairment and evidence of PD pathologic hallmark, SNCA and intra-cytoplasmic inclusion (Xian-Si, Geng and Jin- Jing 2018).Sinemet and salvia officinalis and their combination prevented the increase in MDA levels and decrease in brain anti-oxidant status induced by rotenone treatment. Cell death and reduction in neuron size induced by rotenone was prevented by treatment with sinemet, salvia officinalis and their combination Salvia officinalis has a neuroprotective effect and may help protect the brain from oxidative damage However, breaking through a therapeutic approach that can modify disease progression is very limited to pilot clinical studies or animal experiment (Stoddard-Bennett and Pera 2020)

4.2. Weight

In comparing group 2 (untreated and exposed to rotenone) with group 1 (control), it is found that the former reveals a significant decrease in the rat's weight on day 30 as compared with day 0. Prior to treatment, each rat's weight was assessed, and then every fifteen day after that.Throughout the study, the average weight of the rats in the control group grew. Rats in the rotenone group, on the other hand, lost weight, which was thought to be linked to gastrointestinal neuron damage (E.Drolet, et al. 2009), a matter which disturb digestion. Patients with Parkinson's disease also reported weight loss as a

symptom. The weight loss recorded in this study is consistent with the results published by (Cannon, et al. 2009), and may be caused by the rats' acute inadaptation to the rotenone treatment. When the rats were given rotenone, they became extremely weak and had trouble moving and food intake. They also showed behavioral deficiencies as a result of the treatment, including weight loss, impaired motor abilities, and decreased food intake (Bai, et al. 2016). Early studies linked the depletion in DA level with decreased movement which leads to lesson food consumption and thus causing a decreasing in body weight (Fitzsimmons et al., 2006).In Sinemet group, there was no significant decrease in the weight of day 15 and 30 as compared with day 0. This insignificant decrease in weight is due to the effect of rotenone which is overcome by L-dopa that compensates the diminished DA level, as Selective activation of D1 dopamine receptor (Drd1)-expressing neurons in the prefrontal cortex induces food intake (B.B. Land, N.S. Narayanan,2014). After S. Officinalis treatment, the significant weight reduction is recorded in Parkinson's affected rats on day 30 as compared with day zero for group treated with S. Officinalis only and group of S. Officinalis and sinemet and on day 30 as compared with day 15 of the group of the plant and sinemet . The weight reduction in these groups is higher than that in the rotenone group due to the effect of the rotenone, It was thought to be linked to the damage of gastrointestinal neurons. Moreover, Salvia officinalis L. (sage) leaves have PPAR γ agonistic, pancreatic lipase and lipid absorption inhibitory, suggesting that it may have hypo-lipideamic and anti-oxidant properties, lipid peroxidation inhibitory and anti-inflammatory effects. Inhibition of pancreatic lipase, a key enzyme for fat digestion may have potential as an effective means to alter fat absorption .(Hernandez-Saavedra et al2016) Reported that infusion prepared from this plant reduced serum triglycerides, total cholesterol, and low density lipoproteins (LDL) levels in diet-induced obese rats.26 It also decreased body weight and abdominal fat mass in these animals. In group 5 where sinemet and S. officinalis given concomitantly to the rats the weight significantly decreased in day 15 and 30 as compared with day 0 where the effect of rotenone and S. officinalis overcome the effect of sinemet in increasing the weight.

4. 3. The Behavioral Tests

4. 3. 1. Rotarod Test

In the rotarod apparatus, repeated rat exposure to rotenone significantly reduced the coordination of muscles (rotations number, rotations distance and time of rotations) when put in comparison with the control group which agrees with the previous studies of (Kandil, et al. 2016). The decrease in the DA level is linked to decrease movement in some previous studies (Fitzsimmons et al., 2006).S. Officinalis treatment preserved the coordination of muscles in the rotarod apparatus and improved rotation number, rotation distance and time of rotation as compared to group 2. This indicates that S. Officinalis has a good effect on improving the symptoms of PD in rats especially with a group of salvia officinalis and sinemet, which showed insignificant difference as compared to control group. This finding by the current study hasnotbeen arrived at by previous studies, as far as the researcher could investigate. This improving effect of S. Officinalis is attributed to its powerful neuroprotective impact that has shown an efficient outcome as a neuroprotective agent in other neurodegenerative diseases as Alzheimer's disease because of retaining its anti-inflammatory and antioxidant agents Aqueous extracts of S. officinalis leaf have a high ability to protect the brain and liver homogenates from both Fe(II)- and SNP-induced lipid peroxidation in vitro; this high protective ability of the extract may be due to the antioxidant effect of the high vitamin C and total phenol content of the leaf. However, the main mechanism through which they bring about their protection is by their Fe(II) chelating ability, reducing power, and NO radical scavenging ability, but their OH radical scavenging ability is low. The antioxidant and protective effects of this leaf could be harnessed in the management and prevention of degenerative diseases associated with oxidative stress.(I. Grzegorczyk, A. Matkowski, H. W2007) In sinemet group there was a major progress of the rotarod performance on day 30 was the result of the typical treatment of L-dopa and carbidopa when compared with animals treated with rotenone due to increasing in DA level, which agrees with the study of (Peshattiwara, et al. 2020).

4. 3. 2. The latency of falling time Test and Open Field

Crossing, risk factor, rearing and grooming decreased significantly in group 2 as compared with the other four groups. Exposure to rotenone significantly reduced in the locomotor ability when compared with rats in S. Officinalis group, Sinemet group, S. Officinalis+ sinemet group and control group as evidenced by the decrease in: (1) the total distance travelled in the open field; (2) the no. of visits to central area; (3) the number of rearing; and (4) the time of grooming Rotenone is found to induce significant neuro-muscular dysfunction and motor incompetence in the rats which also demonstrated a shorter time to hang on a beam with less tenacity (Force griping test). Additionally, as seen by the significantly reduced rearing number and grooming time, some rats had an enhanced frozen phase characterized by limited activity. These findings agree with the study of (Farombia, et al. 2019). In addition, the current study had concluded that depression is the most common psychiatric problem in PD The oxidation of DA, a transmitter of DA-ergic neurons, boosts rotenone-induced neurotoxicity in rat SN, resulting in decreased striatal DA levels and reduced motor activity and mobility in rotenone-treated rats. Most of the pathways known to be critical in Parkinson's disease etiology are recapitulated by the rotenone model (Priyanga, Vijayalaksmhi and SelvAraj 2017) .S. Officinalis therapy, on the other hand, enhanced the behavioral deficiency with indication of function recovery(Zineb Choukairi,* Tahar Hazzaz,[2019)]. With decrease of the time of the immobility associated with a significant increase in the time spent in the center of the open field area rearing number, grooming time, hanging time, and minimal rigidity in the treated rats compared to the rotenone group,. S. Officinalis group and S. Officinalis+ sinemet group improved the motor coordination of parkinsonian rats.The behavioral activities of Sinemet group (group 3) In both the open field and force gripping tests, L-dopa-treated rats demonstrated a considerable increase in crossing, risk factor, rearing, grooming and hanging time as compared with rotenone group. These findings agree with (Maniyath, Solaiappan and Rathinasamy 2017).Treatment with L-dopa enhanced the levels of DA and NE, as well as their metabolites, as well as their production and release. L-dopa is the precursor of DA, and it easily passes through the BBB to create DA, which is then converted to NE. As a

result, L-dopa is the main therapy for compensating for decreased DA levels in Parkinson's patients (Shehataa, et al. 2020).

4. 4. Biochemical Parameters

4. 4. 1. Malondialdehyde

Malondialdehyde (MDA) is an accepted marker of lipid oxidative damage. Malondialdehye was produced when highly reactive oxygen metabolites, particularly hydroxyl radicals, act on unsaturated fatty acids of phospholipids components of membranes (Deokar P., Jagtap A., 2016)The present study has found that the rotenone group has shown highly significant increase in the MDA level as compared with the control group which agrees with the study of (Wang, et al. 2020). The development of neurodegenerative disorders is influenced by oxidative stress. Oxidative stress has been associated with the development of PD in both preclinical and clinical investigations especially elevation the concentrations of oxidative markers such as MDA. Similarly, pre-clinical investigations clearly demonstrated that oxidative stress in PD is caused by environmental factors such as neurotoxins, insecticides, pesticides, and DA itself. Pesticides, such as rotenone, have been shown to enhance ROS by blocking mitochondrial complex I activity, resulting in oxidative stress, which may be the cause of SNCA accumulation (Parkhe, et al. 2019). In Parkinson's disease-affected rat groups treated with Salvia officinalis extract revealed a significant reduction in MDA level there is a considerable reduction in MDA levels as compared to the rotenone with respect to lipid peroxidation and anti-oxidant concentration, indicating that the plant has anti-oxidant capacity, Aqueous extracts of S. officinalis leaf have a high ability to protect the brain from both Fe(II)- and SNP-induced lipid peroxidation in vitro; this high protective ability of the extract may be due to the anti-oxidant effect of the high vitamin C and total phenol content of the leaf. However, the main mechanism through which they bring about their protection is by their Fe(II) chelating ability, reducing power, and NO radical scavenging ability, but their OH radical scavenging ability is low. The anti-oxidant and protective effects of this leaf could be harnessed in the management and prevention of degenerative diseases associated with oxidative stress.(I. Grzegorczyk, A. Matkowski, H. W2007).(_Oboh a nd H enle 2009), These results confirmed

that Salvia officinalis can significantly modulate oxidative stress parameters (Amina Boussadia1*, Omar Kharoubi1 2020) Thus, oxidative stress inhibition could be one of the mechanisms underlying S. Officinalis's anti-Parkinson benefits. In Sinemet group, there was also a significant increase in MDA level as compared with control group but less than rotenone group which agrees with the study of (Minelli, et al. 2010). Treatment with L-dopa resulted in a rise in MDA and oxidized GSH levels, in addition to a depletion in reduced GSH. This impact could be explained by the fact that recurrent L-dopa treatment increases DA synthesis, which could lead to an excess of free radical generation, which would overwhelm the endogenous defense mechanism, causing an excess of oxidative stress. Previous investigations have shown that repeated L- dopa administration causes oxidative stress and inflammation, which supports this theory (Teema, Zaitone and Mustafa 2016).

4. 5. Histoimmunochemical study

The decrease in TH due to degeneration of dopaminergic neurons have been reported to play a prominent role in the reduced brain concentrations of dopamine and the manifestations of the clinical motor symptoms in patients with PD (Johnson et al. 2018). The changes in TH expression have been used to show the rate of dopamine turnover or as an indirect measurement of dopaminergic activity, hence could serve as a predictor of the severity or progression of the disease (Tabrez et al. 2012). Consequently, TH enzyme is being viewed as an additional target for identifying new therapeutics for the disease. TH is an enzyme which converts the amino acid tyrosine into dihydroxyphenylalanine This reaction is the first step in the production of dopamine. It is a rate-limiting enzyme that controls the first step of dopamine biosynthesis. The expression of TH is in the right-side brain tissue of SNpc was detected by Western blotting . Lower expression of TH was observed in the right side of SNpc in the rotenone group than in the control group here was a significant reduction in expression of TH immuno-positive neurons in the substantia nigra of rats treated with rotenone. The loss of TH cells and dopaminergic neurons that serves as a local source of dopamine has been reported to be responsible for the motor deficits c a u s e d b y rotenone (A l a m a n d S c h m i d t 2 0 0 2 ; Dhanalakshmi et al. 2016). The IHC DAB

staining images revealed a significant increase in the number of TH-positive cells in the substantia nigra 30 days after sinemet exposure compared to the rotenone group which agrees with the study (Parastoo Zarrin2021) In the present study S. Officinalis cause increase in tyrosine hydroxylase neuron cells as it decreased the destruction of dopaminergic neurons by its mechanisms as antioxidant and anti-inflammatory. The antioxidant potential is directly related to the radical scavenging ability. High levels of radicals can cause far-reaching damage to cellular structures. Antioxidants play an important role in protecting the cells against oxidative damage. Polyphenols(components of salvia officinalis) including flavonoids, belong to a group of natural compounds characterized by strong anti-oxidant properties. the polyphenols and essential oils of Salvia officinalis also possess biological properties such as anti-bacterial , antioxidant, antitumor (C. S. C. Garcia,2016), antinociceptive, and anti-inflammatory activities and cytotoxic and cytogenetic effects (R. K. Al-Barazanjy, 2013). As a results of several studies suggest that S. officinalis has a power anti-oxidant activity. (e phenolic compounds are isolated from the extract of Salvia officinalis such as carnosol, rosmarinic, and carnosic acids, followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin with the most effective antioxidant activity (. Al-Asadye and M. Esmaeilizadeh 2017). In addition, rosmarinic acid and flavonoids of S. officinalis especially quercetin and rutin have stronger antioxidant activity (M. I. Azevedo, 2013).(Zakaria Khiya , 1, 2 Yassine Oualcadi,2021) in group 5 there was more increment in TH + ve neurons refering to the synergistic activity of each S. officinalis and sinemet Immunohistochemcial data showed that there is obvious difference in intensity of stain between all groups , rotenone group show low intensity of stain as it significantly increased the loss of dopaminergic neurons in the substantia nigra,(H. score= 198.84) and decreased the striatal expression of tyrosine hydroxylase. Further, rotenone administration activated microglia and astroglia, which in turn up regulated the expression of α-synuclein, pro-inflammatory, and oxidative stress factors, resulting in PD pathology. While in sinemet group we noticed that threre is enhancement in the stain because significant increase in H. score (H.S.= 250.756) showing neuroprotective effects that prevented TH-positive neuronal loss (Leilei Chen, Yujv

Huang2021) In S. officinalis groupthe intensity of stain better than rotenone group but slightly less than group 3 and group 5, the H. score was (H.S. = 235.65) releaving the activity of S. officinalis as anti-oxidant and inti-inflammatory in preventing loss of the dopaminergic neurons. In group 5 (H.S. = 255.0285) the stain was significantly more than groups 2,3,4 where sinemet and S.officinalis synergistically work together to preserve the dopamimergic neurons in substantia nigra and decrease the oxidative stress and preventing loss of tyrosine hydroxylase

Chapter Five

Conclusion and Recommendations

Conclusion

1. S. officinalis improves the motor activity in the rotenone - induced Parkinson disease in male rats.
2. S. officinalis potentiates the effect of sinemet in rotenone induced Parkinson disease in male rats.
3. S. officinalis, is effective in decreasing the level of MDA in the tissues which indicates its effective role as an anti-oxidant agent in rotenone induced Parkinson disease in male rats.
4. S. officinalis, is effective in increasing the levels of tyrosine hydroxylase in brain tissues in the rotenone - induced Parkinson disease in male rats.

Recommendations

1. Further studies are needed for identification of major active constituents of S. officinalis.

2. More studies needed to determine the effects of S. officinalis on inflammatory mediators such as cytokines: IL-6, IL-1B in neurodegenerative diseases.

3. Clinical trials are needed to study the effect of S. officinalis on patients with Parkinson disease.

Chapter six

References

Abdelkadera, Noha F., Heba A. Faridb, Eman R. Younessc, Omar M.E. Abdel-Salamb, and Hala F. Zakia. "The Role of KATP Channel Blockade and Activation in the Protection against Neurodegeneration in the Rotenone Model of Parkinson's disease." Life Sciences , july 13, 2020 : 1-9.

Akira Nakashima , Akira Ota , Yoko S. Kaneko , Keiji Mori , Hiroshi Nagasaki ,Toshiharu Nagatsu. A possible pathophysiological role of tyrosine hydroxylase in Parkinson's disease suggested by postmortem brain biochemistry 9 May 2012

Al-Asadye and M. Esmaeilizadeh, "Pharmacological properties of Salvia officinalis and its components," Journal of Traditional and Complementary Medicine, vol. 7, no. 4, pp. 433–440, 2017.

Alabi, Akinyinka O., Abayomi M. Ajayi, Benneth Ben-Azu, Osarume Omorobge, and Solomon Umukoro. "Methyl Jasmonate Ameliorates Rotenone-Induced Motor Deficits in Rats Through its Neuroprotective Activity and Increased Expression of Tyrosine Hydroxylase Immunopositive Cells." Metabolic Brain Disease, August 28, 2019 ;34(6):1723-1736.

Alcaro A, Huber R, Panksepp J (December 2007). "Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective". Brain Research Reviews. 56 (2): 283–321.

Amina Boussadia, Omar Kharoubi, Zakia Lahouel, Abderrezzak Benglia, Abdelkader Aoues, Effect of aqueous Salvia officinalis extract on Aluminum chloride-induced neurotoxicity in female rats International Journal of Pharmaceutical Research & Allied Sciences, 2020, 9(2):139-150

Anusha C, Sumathi T, Joseph LD. "Protective role of apigenin on rotenone induced rat model of Parkinson's disease: Suppression of neuroinflammation and oxidative stress mediated apoptosis". Chem Biol Interact, May 1, 2017: 269:67-79.

Aremu, Olukayode O., Adebola O. Oyedeji, Opeoluwa O. Oyedeji, and Benedicta N. Nkeh-Chungag and Constance R. Sewani Rusike. "In Vitro and In Vivo Antioxidant Properties of Taraxacum offcinale in N-Nitro-l-Arginine Methyl Ester (L-NAME)-Induced Hypertensive Rats." Antioxidants, August 15, 2019 :1-12.

Aid S, Bosetti F (2011) Targeting cyclooxygenases-1 and -2 in neuroinflammation: therapeutic implications. Biochimie.93:46–51.

Agostinho P, Cunha RA, Oliveira C (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des 16:2766–2778

Brahadeeswaran, Subhashini [1] ; Lateef, Mohammad [2] ; Calivarathan, Latchoumycandane [1] ; An Insight into the Molecular Mechanism of Mitochondrial Toxicant-induced Neuronal Apoptosis in Parkinson's Disease. Current Molecular Medicine 2022.

Berg D, Lang AE, Postuma RB, et al. Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. Lancet Neurol 2013;12(5):514–524.

Barbara S. Connolly, A. Lang Medicine, Psychology , Pharmacological treatment of Parkinson disease: a review. JAMA 2014

Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. Front Neuroanat, 2015; 9: 91.

Bai, Qunhua, Junlin He, Yong Tang, Shibo Wang, Jingfu Qiu, et al, "Rotenone-Induced Energy Stress Decompensated in Ventral Mesocerebrum is Associated with Parkinsonism Progression in Rats." Experimental and Theraputic Medicine, May 18, 2016:1060-1066.

Breckenridge CB, Berry C, Chang ET, et al. Association between Parkinson's disease and cigarette smoking, rural living, well-water consumption, farming and pesticide use: systematic review and meta-analysis. PLoS One 2016; 11: e0151841.

B.B. Land, N.S. Narayanan, R.J. Liu, C.A. Gianessi, C.E. Brayton, D.M. Grimaldi, M. Sarhan, D.J. Guarnieri, K. Deisseroth, G.K. Aghajanian, R.J. DiLeone, Medial prefrontal D1 dopamine neurons control food intake Nat. Neurosci., 17 (2014), pp. 248-253

Braak H, Del Tredici K, R€ub U, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003; 24: 197–211.

C. S. C. Garcia, C. Menti, A. P. F. Lambert et al., "Pharmacological perspectives from Brazilian Salvia officinalis(Lamiaceae): antioxidant, and antitumor in mammalian cells," Anais da Academia Brasileira de Ci^encias, vol. 88, no. 1, pp. 281–292, 2016.

Cannon R., Jason, VictorTapias, Hye MeeNa, Anthony S.Honick, Robert E.Drolet, and J. TimothyGreenamyre. "A Highly Reproducible Rotenone Model of Parkinson's disease." Neurobiology of Disease, May 2009: 279-290.

Cerri, S.; Mus, L.; Blandini, F. Parkinson's Disease in Women and Men: What's the Difference? J. Parkinsons Dis. 2019, 9, 501–515.

Carroll Rutherford Fields, Nora Bengoa-Vergniory, and Richard Wade-Martins. (2019). REVIEW ARTICLE Targeting Alpha-Synuclein as a Therapy for Parkinson's Disease. Front. Mol. Neuroscience.

Chang, Kuo-Hsuan, and Chiung-Mei Chen. "The Role of Oxidative Stress in Parkinson's Disease." Antioxidants, July 8, 2020: 1-31

Dorsey ER , Sherer T , Okun MS , Bloem BR The emerging evidence of the Parkinson pandemic. J Parkinsons Dis. 2018; 8: S3-S8

Darweesh SK, Koudstaal PJ, Ikram MA. Trends in the incidence of Parkinson disease. JAMA Neurol 2016; 73: 1497

Dugger BN, Adler CH, Shill HA, et al. Concomitant pathologies among a spectrum of parkinsonian disorders. Parkinsonism Relat Disord. 2014;20:525–9.

Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinsons Dis., 2013; 3(4): 461–91.

Devos, D., Lejeune, S., Cormier-Dequaire, F., Tahiri, K., Charbonnier-Beaupel, F., Rouaix, N., et al. (2014). Dopa-decarboxylase gene polymorphisms affect the motor response to L-dopa in Parkinson's disease. Parkinsonism Relat. Disord. 20, 170–175.

Drolet E, Robert, Jason R.Cannon, LauraMontero, and J. Timothy Greenamyre. "Chronic Rotenone Exposure Reproduces Parkinson's Disease Gastrointestinal Neuropathology." Neurobiology of Disease, October 2009, 36 ed.: 96-102.

Deokar P., Jagtap A., and Yerawar C.(2016) Correlation of protein carbonyl and MDA in diabetes and its complications. Indian Journal of Basic and Applied Medical Research; 5(2):284-289

Delamarre A, Meissner WG. (2017). Epidemiology, environmental risk factors and genetics of Parkinson's disease. Presse Med. 2017;46:175–81.

Dhanalakshmi C, Janakiraman U, Manivasagam T, Justin TA, Essa MM, Kalandar A, Khan MA, Guillemin GJ (2016) Vanillin attenuated behavioural impairments, neurochemical deficts, oxidative stress and apoptosis against rotenone induced rat model of Parkinson's disease. Neurochem Res 41:1899–1910

Dent, M.; Dragovi´c-Uzelac, V.; Peni´c, M.; Brñi´c, M.; Bosiljkov, T.; Levaj, B. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (Salvia officinalis L.) extracts. Food Technol. Biotechnol. 2013, 51, 84–91.

El-Kholy W.M, El-Habibi E.M and Mous A.T (2010). Oxidative stress in brain of male rats intoxicated with aluminum and the neuromodulating effect of forms of sage (Salvia officinalis). Journal of American Science; 6(12): 1283-1297.

Eikelenboom P, Van Exel E, Hoozemans JJ, Veerhuis R, Rozemuller AJ, Van Gool WA (2010) Neuroinflammation—an early event in both the history and pathogenesis of Alzheimer's disease. Neurodegener Dis 7:38–41

Fritsch T, K Smyth, M Wallendal, T Hyde, G Leo, D Geldmacher: Parkinson Dis ease: Research update and clinical management. Southern Medical Association 105(12), 650-656 (2012)

Farombia EO, Awogbindin IO, Farombi TH, Oladele JO, Izomoh ER, et al. "Neuroprotective Role of Kolaviron in Striatal Redo- Inflammation Associated with Rotenone Model of Parkinson'sDisease." Neurotoxicology, March 28, 2019 : 132–141.

Fitzsimmons F., Dominick, C.Moloney, Teresa and Dowd, Eilís. "Further validation of the corridor task for assessing deficit and recovery in the hemi Parkinsonian rat: Restoration of bilateral food retrieval by dopamine receptor agonism." Behavioural Brain Research. 169, May 15, 2006, 2, pp. 352-355.

Fox SH, Katzenschlager R, Lim S-Y, et al. International Parkinson and Movement Disorder Society evidence-based medicine review: update on treatments for the motor symptoms of Parkinson's disease. Mov Disord 2018;33(8):1248–66.

Gillies GE, Pienaar IS, Vohra S, et al. Sex differences in Parkinson's disease. Front Neuro-endocrinol 2014; 35: 370–84.

Generalić I, Skroza D, Šurjak J, Možina SS, Ljubenkov I, Katalinić A, Šimat V, Katalinić V. Seasonal variations of phenolic compounds and biological properties in sage (Salvia officinalis L.). Chemistry & biodiversity. 2012;9(2):441-57.

Genaro Gabriel Ortiz, Fermín P. Pacheco-Moisés,Mario A. Mireles Ramírez, L. Javier Flores-Alvarado, Héctor González-Usigli, Angélica L. Sánchez-López, Lorenzo Sánchez-Romero, Irma E. Velázquez-Brizuela, Erika Daniela González-Renovato and Erandis Dheni Torres-Sánchez .Oxidative Stress and Parkinson's Disease: Effects on Environmental Toxicology.2016

Greenamyre, Terina N. Martinez and J. Timothy. "Toxin Models of Mitochondrial Dysfunction in Parkinson's Disease." Antioxidants & Redox Signaling, 2012

Hunot S., Hirsch EC. Neuroinflammatory processes in Parkinson's disease. Ann Neurol. 2003;53(suppl 3):S49–S58.

Harry GJ, Kraft AD (2008) Neuroinflammation and microglia: considerations and approaches for neurotoxicity assessment. Expert Opin Drug Metab Toxicol 4:1265–1277

Hammad Ullah and Haroon Khan. Anti-Parkinson Potential of Silymarin: Mechanistic Insight and Therapeutic Standing.2018

H€oglinger GU, Michel PP, Champy P, et al. Experimental evidence for a toxic etiology of tropical parkinsonism. Mov Disord 2005; 20: 118–9.

Hernandez-Saavedra D, Perez-Ramirez IF, Ramos-Gomez M, Mendoza-Diaz S,Loarca-Pina G, Reynoso-Camacho R. Phytochemical characterization and effect of Calendula officinalis, Hypericum perforatum, and Salvia officinalis infusions on obesity associated cardiovascular risk. Med Chem Res. 2016;25: 163e172

Huot, P., Fox, S. H., and Brotchie, J. M. (2015). Monoamine reuptake inhibitors in Parkinson's disease. Parkinsons. Dis. 2015:609428. doi: 10.1155/2015/ 609428.

I. Grzegorczyk, A. Matkowski, H. Wysoki ńska, Antioxidant activity of extracts from in vitro cultures of Salvia officinalis L., Food Chem. 104 (2007) 536–541

Juarez Olguin, H., Calderon Guzman, D., Hernandez Garcia, E., and Barragan Mejia, G. (2016). The Role of dopamine and its dysfunction as a consequence of oxidative stress. Oxid. Med. Cell. Longev. 2016:9730467.

Javed, Hayate, Sheikh Azimullah, M. EmdadulHaque, and Shreesh K. Ojha. "Cannabinoid Type2 (CB2) Receptors Activation Protects against Oxidative Stress and Neuroinflammation Associated Dopaminergic Neurodegenerationin Rotenone Model of Parkinson's Disease." Frontiers in Neuroscience, August 2, 2016: 1-14.

Johnson, M. E., Stecher, B., Labrie, V., Brundin, L., and Brundin, P. (2019). Triggers, facilitators, and aggravators: redefining Parkinson's disease pathogenesis. Trends Neurosci. 42, 4–13.

Jankovic, Joseph, I Hurtig Howard, and F Eichler April. "Etiology and Pathogenesis of Parkinson Disease." UpToDate, February 9, 2021

Johnson IME, Salvatore MF, Maiolo SA, Bobrovskaya L (2018) Tyrosine hydroxylase as a sentinel for central and peripheral tissue responses in Parkinson's progression: evidence from clinical studies and neu-rotoxin models. ProgNeurobiol 165–167:1–25

Kristian Jensen, RikkeKrusenstjerna-Hafstrøm, JesperLohse, Kenneth H Petersen & Helene Derand .(2017). A novel quantitative immunohistochemistry method for precise protein measurements directly in formalin-fixed, paraffin- embedded specimens: analytical performance measuring HER2 .Modern Pathology volume 30, pages180–193

Kandil, Esraa A., Noha F. Abdelkader, Bahia M. El-sayea, and Samira Saleh. "Imipramine and Amitriptyline Ameliorate the Rotenone Model of Parkinson's Disease in Rats." Neuroscience, 2016: 26–37.

Kumar, Puneet, Harikesh Kalonia, and Anil Kumar. "Possible GABAergic Mechanism in the Neuroprotective Effect of Gabapentin and Lamotrigine against 3-Nitropropionic Acid Induced Neurotoxicity." Eur J Pharmacol, Jan 2012.

Lim SY, Lang AE. The nonmotor symptoms of Parkinson's disease: an overview. Mov Disord. 2010;25:S123–30.

LeWitt P.A. , Fahn S. Levodopa therapy for Parkinson disease: a look backward and forward. Neurology. 2016; 86: S3-S12

Lee A, Gilbert RM. Epidemiology of Parkinson disease. Neurol Clin 2016; 34: 955–65

Li, D., Liu, F., Yang, T., Jin, T., Zhang, H., Luo, X., et al. (2016). Rapamycin protects against neuronal death and improves neurological function with modulation of microglia after experimental intracerebral hemorrhage in rats. Cell Mol. Biol. (Noisy-le-grand) 62, 67–75.

Leilei Chen *, Yujv Huang , Xing Yu , Jiahong Lu , Wenting Jia , Juxian Song ,Liangfeng Liu , Youcui Wang , Yingyu Huang , Junxia Xie * and Min Li. Corynoxine Protects Dopaminergic Neurons Through Inducing Autophagy and Diminishing Neuroinflammation in Rotenone-Induced Animal Models of Parkinson's Disease. April 2021

Miyazaki, I., Isooka, N., Imafuku, F., Sun, J., Kikuoka, R., Furukawa, C., et al. (2020). Chronic systemic exposure to low-dose rotenone induced central and peripheral neuropathology and motor deficits in mice: reproducible animal model of Parkinson's disease. Int. J. Mol. Sci. 21, 3254.

MarikaCordaro, Salvatore Cuzzocrea, and RosaliaCrupi.(2020). An Update of Palmitoylethanolamide and Luteolin Effects in Preclinical and Clinical Studies of Neuroinflammatory Events. Antioxidants, 9, 216;

Mbiydzenyuy, Ngala Elvis, Herbert Izo Ninsiima, Miriela Betancourt Valladares, and Anatole Piemecor. "Zinc and linoleic acid pre-treatment attenuates biochemical and histological changes in the midbrain of rats with rotenone-induced Parkinsonism." BMC Neuroscience, May 9, 2018.

Martina Jakovljevi´c, Stela Joki´c,* Maja Molnar , Midhat Jaši´c, Jurislav Babi´c, Huska Juki´c and Ines Banjari. Bioactive Profile of Various Salvia officinalis L. Preparations. March 2019

Miura K, Kikuzaki H, Nakatani N. Antioxidant activity of chemical components from sage (Salvia officinalis L.) and thyme (Thymus vulgaris L.) measured by the oil stability index method. J Agric Food Chem; 2002; 50(7): 18, 45-51.

Mittal S, Bjørnevik K, Im DS, et al. b2-adrenoreceptor is a regulator of the a-synuclein gene driving risk of Parkinson's disease. Science 2017; 357: 891–8.

Minelli, Alba, Conte C, Prudenzi E, Cacciatore I, Cornacchia C, Taha E, Pinnen F. "N-acetyl-L-methionyl-L-Dopa-methyl Ester as a Dual Acting Drug that Relieves L-Dopa-induced Oxidative Toxicity."

Free Radic Biol Med, Mar 20, 2010; 49(1):31-9.

Maniyath, Sukala Puthuparambil, Narayanan Solaiappan, and Muthusamy Rathinasamy. "Neurobehavioural Changes in a Hemiparkinsonian Rat Model Induced by Rotenone." J Clin Diagn Res, National Library Medicine, MAR 11, 2017.

Niranjan R (2014) The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Mol Neurobiol 49:28–38

Nefeli S. Sotiropoulou , Stiliani F. Megremi and Petros Tarantilis * Evaluation of Antioxidant Activity, Toxicity, and Phenolic Profile of Aqueous Extracts of Chamomile (Matricaria chamomilla L.) and Sage (Salvia officinalis L.) Prepared at Different Temperatures. March 2020

Nishijima H, Ueno T, Funamizu Y, Ueno S, Tomiyama M (2018) Levodopa treatment and dendritic spine pathology. Mov Disord 33:877–888

Nigel Bruce , Daniel Pope , Debbi Stanistreet. (2018). Quantitative Methods for Health Research: A Practical Interactive Guide to Epidemiology and

Statistics , 2nd Edition. ISBN-13: 978-1118665411. ISBN-10: 9781118665411. Publisher: Wiley- Blackwel.

Oboh, G. and Henle, T. (2009). Antioxidant and inhibitory effects of aqueous of Salvia officinalis leaves on pro- oxidant- induced lipid peroxidation in brain and liver in vitro. Medicinal Food, 12: 77 -84.

Poewe W. , Seppi K. , Tanner C.M. , et al.Parkinson disease. Nat Rev Dis Prim. 2017; 3: 1-21

P. Capek, E. Machová, J. Turjan, Scavenging and antioxidant activities of immuno modulating polysaccharides isolated from Salvia officinalis L., Int. J. Biol. Macromol.44 (2009) 75–80

Priyanga, K. Srimarhi, K. Vijayalaksmhi, and R. SelvAraj. "Behavioral Studies of Wistar Rats in Rotenone Induced Model of Parkinson's Disease." International Journal of Pharmacy and Pharmaceutical Sciences, Sep 21, 2017, 9 ed.: 975-1491.

Parastoo Zarrin1 · Mahmood Dehghani Ashkezari1 · Seyed Morteza Seifati1

Liposomal Form of L-Dopa and SH-Sy5y Cell-Derived Exosomes Modulate the Tyrosine Hydroxylase/Dopamine Receptor D2 Signaling Pathway in Parkinson's Rat Model : 28 April 2020

Przedborski, S. (2017). The two-century journey of Parkinson disease research. Nat. Rev. Neurosci. 18, 251–259.

Rocha EM, De Miranda B, Sanders LH. Alpha-synuclein: pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. Neurobiol Dis2018; 109: 249–57.

Rustay, Nathan R., Douglas Wahlsten, and John C. Crabbe. "Influence of Task Parameters on Rotarod Performance and Sensitivity to Ethanol in Mice." Behavioural Brain Research, 2003, 141 ed.: 237-249.

R. K. Al-Barazanjy, K. Dizaye, and A. Al-Asadye, "Cytotoxic and cytogenetic effects of salvia officinalis on different tumor cell lines,"

Middle East Journal of Internal Medicine, vol. 6, no. 4, pp. 15–25, 2013.

Shehataa, Ahmed M., Omar A. Ahmed-Farida, Hanan A. Rizkb, Sara M. Saberc, Fawzy M. Lashind, and Lamberto Ree. "Neurochemical, Neurobehavioral and Histochemical Effects of Therapeutic Dose of L-dopa on Striatal Neurons in Rats: Protective Effect of Virgin Coconut oil." Biomedicine & Pharmacotherapy, June 24, 2020.

Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. Nat Rev Neurosci 2017; 18: 435–50.

Sampson TR, Debelius JW, Thron T, et al. Gut micro-biota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell 2016; 167: 1469–1480.e12

Sharma, Shakshi, Khadga Raj, and Shamsher Singh. "Neuroprotective Effect of Quercetin in Combination with Piperine Against Rotenone- and Iron Supplement–Induced Parkinson's Disease in Experimental Rats." Neurotoxicity Research, September 25, 2019.

shereen m. mekkey1*, ahmed rahmah abu raghif2, haider abdul ridha alkafaji3, najah r. hadi4 . The anti -Parkinson effects of Liraglutide in rat model of Rotenone induced Parkinsonism. 2020

S. Kianbakht,1* B. Abasi,2 M. Perham3 and F. Hashem Dabaghian4 Antihyperlipidemic Effects of Salvia officinalis L. Leaf Extract in Patients with Hyperlipidemia: A Randomized Double-Blind Placebo-Controlled Clinical Trial , 25: 1849–1853 (2011)

Teive HA, Munhoz RP. Postural instability in Parkinson's disease—120 years after Charcot's death. Arq Neuropsiquiatria, 2014; 72: 633-35

Theo Stoddard-Bennett and Renee ReijoPera. (2018). Review Treatment of Parkinson's Disease through Personalized Medicine and Induced Pluripotent Stem Cells, The Molecular and Cellular Basis of Neurodegenerative Diseases Chapter 1 - Solving the Puzzle of Neurodegeneration,

Teema, Asmaa M, Sawsan A Zaitone, and Yasser M Moustafa. "Ibuprofen or Piroxicam Protects Nigral Neurons and Delays the Development of L-dopa Induced Dyskinesia in Rats with Experimental Parkinsonism: Influence on Angiogenesis." Neuropharmacology, Aug 2016: 432-450.

Tabrez S, Jabir NR, Shakil S, Greig NH, Alam O, Abuzenadah AM, Damanhouri GA, Kamal MA (2012) A synopsis on the role of tyrosine hydroxylase in Parkinson's disease. CNS Neurol Disord Drug Targets 11:395–409

von Campenhausen S, Bornschein B, Wick R, et al. Prevalence and incidence of Parkinson's disease in Europe. Eur Neuropsychopharmacol J 2005; 15: 473–90.

Wang, Tian, Cuiting Li, Bing Han, Zhenhua Wang, Xiaoyu Meng, Leiming Zhang,et al. "Neuroprotective effects of Danshensu on Rotenone-Induced Parkinson's Disease Models in Vitro and in Vivo." BMC Complementary Medicine and Therapies , January 23, 2020.

Werner Poewe, MD , Philipp Mahlknecht, MD, PhD Pharmacologic Treatment of Motor Symptoms Associated with Parkinson Disease 2020

Zineb Choukairi,1,* Tahar Hazzaz,1 Mustapha Lkhider,1 Jose Manuel Ferrandez,2 and Taoufiq Fechtali, Effect of Salvia Officinalis L. and Rosmarinus Officinalis L. leaves extracts on anxiety and neural activity 2019 Mar 15

Zakaria Khiya , Yassine Oualcadi, Abderrahmane Gamar,Fatima Berrekhis, Touria Zair, and Fatima EL Hilali. Correlation of Total Polyphenolic Content with Antioxidant Activity of Hydromethanolic Extract and Their Fractions of the Salvia officinalis Leaves from Different Regions of Morocco. February 2021

الخلاصة

مرض باركنسون هو مرض مستعصي ينتج من تحلل الاعصاب الدوبامينية موضعيا في المادة السوداء في الدماغ، عادة التحلل الانتقائي للاعصاب الدوبامينية و ظهور اجسام ليوي في المادة السوداء و الذي هو المكون الاساسي لتجمعات الفا سينكولين في الاعصاب الدوبامينية المتبقية .. تعتبر السمات المرضية الرئيسية لمرض الباركنسون تهدف هذه الدراسة الى تقييم تاثير عشبة المريمية على دماغ نموذج الحيوان لمرض باركنسون بسبب المعرفة المقدمة من الدراسات السابقة حول خصائص نبات السالفيا أوفيسيناليس (المريمية)كمضادات الأكسدة ومضادات الالتهاب، وما إذا كان يمكن أن يظهر نتائج أفضل عند إعطائه بالتآزر مع علاجات باركنسون الأخرى، . تم تقسيم خمسين ذكور جرذ إلى خمس مجموعات ، المجموعة ١ (مجموعة التحكم) لا تتعرض للروتينون ولا تتلقى أي علاج فقط بمحلول ملحي طبيعي لمدة ٣٠ يومًا ، المجموعة ٢ تم حقنها بالروتينون (٢,٥ مجم / كجم) IP لمدة ٣٠ يومًا دون أي علاج، المجموعة ٣ حيث تم حقنها بالروتينون (٢,٥ مجم / كجم) وتلقي قرص سينيميت فمويا بعد تذويبها بالماء في اليوم ١٥ لمدة ٣٠ يومًا أيضًا ، المجموعة ٤ حيث تم حقنها بالروتينون (٢,٥ مجم / كجم) وتلقي مستخلص المريمية (٥٠٠ مجم / كجم) فمويا في اليوم ١٥ ولمدة ٣٠ يوم، المجموعة ٥ حيث يتم حقنها بالروتينون (٢,٥ مجم / كجم) وتلقي مستخلص المريمية (٥٠٠ مجم / كجم) فمويا وتلقي قرص سينيميت فمويا بعد تذويبها بالماء في اليوم ١٥ لمدة ٣٠ يومًا أيضًا.. ثم تم تقييم اداء الجرذان باستخدام اختبار المجال المفتوح و اختبار امساك القوة و اختبار جهاز الروتارود، في نهاية التجارب تم التضحية بالجرذان بطريقة قطع الراس وتم قياس مستوى ال MDA بواسطة enzyme linked immunosorbent assays kits(Elisa) بدم الجرذان وكذلك تم قياس عدد الخلايا العصبية التي تبقى محافظة على انزيم tyrosine hydroxylase وشدة هذا الانزيم بتلك الخلايابواسطة ال immunohistochemical studies على انسجة دماغ الجرذان . في المجاميع ٢ ، ٤، ٥ ، كان هناك نقصان واضح بالوزن مقارنة بالمجموعة ١ و في المجاميع ٢، ٣، ٤، كان هناك نقصان حاد في عدد مرات الدوران و مسافة الدوران ووقت الدوران مقارنه بالمجموعة ١ ، بينما في المجاميع ٣، ٤، ٥ كان هناك زيادة واضحة مقارنة مع مجموعة ٢ في اختبار rotarod test (p value<0.05) في المجاميع ٢، ٣، ٤ كان هناك نقص حاد في المشي او العبور ، الاستمالة والتربية و عدد مرات الذهاب الى وسط المنطقة مقارنة مع مجموعة ١ بينما في المجاميع ٣، ٤، ٥ كان هناك زيادة حادة مقارنة مع المجموعة في اختبار الـ (p value<0.05) open field test بالاضافة لذلك في المجاميع ٢، ٣، ٤ كان هناك نقص حاد في قوة الامساك مقارنة مع مجموعة ١، بينما في المجاميع ٣، ٤، ٥ كان هناك زيادة ملحوظة مقارنة مع مجموعة ٢ في اختبارالـ

force gripping test (p value<0.05) في الدراسات البايوكيميائية كان هناك زيادة ملحوظة في مستوى ال MDA بمجموعة ٢ مقارنة مع مجموعة ١ ، وايضا كان هنالك نقصان ملحوظ في مستواه عند المجاميع ٣، ٤، ٥ مقارنة مع مجموعه ٢(p value<0.05) في ال immunohistochemical studies عدد الخلايا الدوباميئية التي تحتوي على انزيم ال tyrosine hydroxylase اقل بشكل ملحوظ في المجاميع ٢، ٣، ٤، ٥ مقارنة مع مجموعه ١ بينما كان هناك زيادة ملحوظة في المجاميع ٣، ٤، ٥ مقارنة مع المجموعة ٢ (p value<0.05) وكانت شدة الاعصاب الحاوية على هذا الانزيم تقل بشكل ملحوظ في المجاميع ٢، ٣، ٤، ٥ مقارنة مع مجموعه ١ و كان هناك زيادة ملحوظة في المجاميع ٣، ٤، ٥ مقارنة مع مجموعة ٢ .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جـــــامعة بابـــــل/ كليـــــة الطب

تأثير مستخلص نبات المريمية (*Salvia officinalis*) في علاج مرض

الباركنسون المستحث في الجرذان

رسالة مقدمه الى مجلس كلية الطب/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في الأدوية/الأدوية و السموم

من قبل

زينب عليوي صبيح

بكالوريوس صيدلة

(2014-2015)

اشراف

الاستاذ المساعد

الدكتور سلمان محمد سلمان

دكتوراه في علم الادوية

الاستاذ المساعد

الدكتورة اسماء محمد مكي

دكتوراه في علم الانسجة

١٤٤٣ هـ

٢٠٢٢ م