Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Study the neuroprotective effects of ethanolic extract of *Borago.officinalis* flowers on animal model of Parkinson's disease and SH-SY5Y cell line

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

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

صدق اللَّهُ العظيم

سورة المجادلة آية 11

Supervision Certificate

We certify that this thesis entitled (**Study the neuroprotective effects of ethanolic extract of *Borago.officinalis* flowers on animal model of Parkinson's disease and SH-SY5Y cell line**) was prepared under our supervision in the department of pharmacology as a partial fulfillment for the master degree of Pharmacology and Toxicology.

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We certify that we have read this thesis entitled "(**Study the neuroprotective effects of ethanolic extract of *Borago.officinalis* flowers on animal model of Parkinson's disease and SH-SY5Y cell line**)" and as an examining committee examined the student "**Hala Saad Abed**" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master, in Pharmacology and Toxicology.

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Acknowledgements

Primarily I would thank Allah the Most Merciful for being able to complete this project with success.

Then I would like to thank my supervisors **Dr. Selman Mohammed Selman & Dr. Kaiser Neama Madlum**, whose valuable guidance has been the ones that helped me patch this project and make it with that success. Their suggestions and instructions have served as the major contributors towards the completion of the project.

Special thanks and gratitude to the Department of Pharmacology for the infinite support and cooperation, and thanks to all the people who have supported me in completing the research work directly or indirectly.

Finally, All words cannot suffice to thank my lovely family, my brothers, and sisters, and my close friends, for their patience, support, and encouragement….

Hala Saad Abed Madhi

Summary

Parkinson’s disease (PD) is the most common neurodegenerative disease after Alzheimer's. It is characterized by akinesia, uncontrollable shaking, stiffness, postural instability, and tremor. The two main pathogenic processes that cause PD are oxidative stress and neuroinflammation which have been firmly linked to the death of dopaminergic neurons in the substantial nigra (SN) leading to depletion of striatal dopamine (D).

Rotenone (ROT), a neurotoxin that selectively destroys dopaminergic neurons, and causes PD-like symptoms in rats, is used to induce PD.

The aim of this study was to assess the effects of ethanolic extract of *Borago.officinalis* in (ROT)-induced PD models in SH-SY5Y cells and in rats.

In vitro

SH-SY5Ycell lines were seeded and growing in 96 tissue culture plates for 24 hours, then the cells were pretreated with different concentrations of ethanolic extract of *B.officinalis* at serial dilutions ranging from (31.25 to 500 µg/ml) and L-dopa/Carbidopa (10 µg/ml), along with untreated cells as a control group and incubated for 2 hours at 37°C , then treated with ROT (20 µg/ml), and after a 48-hour incubation period following the exposure period, the supernatants were taken for biochemical tests to determine the concentrations of MDA, IL-1β and TAOC Colorimetric assay.

The following results were obtained in vitro:

B.officinalis extract at concentrations (125 and 500 μg/ml) show a significant decreased (*p-value<0.05)* in MDA and IL-1β levels and a significant increased (*p-value<0.05)* in TAOC levels as compared with ROT group.

In Vivo

In this experimental study that extended over 3 months, and about 70 healthy Albino male rats used , divided equally into the following seven groups: the first is the healthy, untreated control group, while the remaining six groups received (ROT) 2.5 mg/kg intraperitoneally (IP) from day to day for 21 days, the second group received (ROT) only, While the third group received an oral dose of (10mg/kg) (L-dopa/Carbidopa (L/C) daily, and the fourth, fifth, sixth and seventh groups received oral doses of (62.5,125,250 and 500 mg/kg) respectively of *B.officinalis* extract (BOE) every day. Neurobehavioral analysis were done via rotarod, open field, and force gripping tests on day 22. Then, after the animals were sacrificed, the heads had been decapitated, samples of brain tissue were prepared for homogenization in order to reach tissue supernatant, which were then used in biochemical tests to determine the concentrations of MDA, IL-1β and TAOC Colorimetric assay.

The following results were obtained in rats:

(1) Results of rotarod apparatus's ability to coordinate rats' muscles showed a significant increase in rotation number, rotation distance and time of rotation in (BOE) treatment doses of (125,250 and 500 mg/kg), as compared with the ROT group .

(2) *B.officinalis* extract at concentrations (125,250 and 500 mg/kg) improved the coordinated movement of the rats having Parkinson's disease with considerable elevations in crossing distance, rearing number, grooming time, hanging time and minimal rigidity.

(3) In rats treated with (BOE) at concentrations (125,250 and 500 mg/kg), show a significant decreased (*p-value <0.05)* in MDA and IL-1β levels and a significant increased (*p-value <0.05*) in TAOC levels as compared to the ROT group.

This indicates that (BOE) effectively alleviates PD symptoms in rats, particularly at *B.officinalis* extract concentration (125 mg/kg).

In conclusion, (BOE) contains antiparkinson's -like activity that can both in vitro and in vivo reverse PD induced by rotenone. These activity involve anti-inflammatory and antioxidant properties.

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List Of Abbreviations

Abbreviations	Meaning
AB	Antibody
ABTS	2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BOE	*Borago. officinalis* Extract
BuChE	Butyrylcholinesterase
B.W	Body Weight
C2H2	Methylene
Ca+2	Calcium ion
c-Ab1	Certain abelson tyrosine kinase
CAT	Catalase
CNS	Central nervous system
COMT	Catecholamine omethyl transferase
COX-2	Cyclooxygenase-2
CYP450	Cytochrome p450
D.W	Distilled water
DDW	Distilled deionized water
DA	Dopamine

List Of Abbreviations

DA-ergic	Dopaminergic
DOPA	Dihydroxylphenylalanine
DJ-1	Protein/nucleic acid deglycase DJ-1
DMSO	Dimethyl sulfoxide solution
DOPAC	Dihyroxyphenylacetic acid
Fe+2	Ferrous iron
Fe+3	Ferric iron
FBS	Fetal Bovine Saline
GLA	Gamma linolenic acid
GPX	Glutathione peroxidase
GSH	Glutathione
H+	Proton
H2O2	Hydrogen peroxide
HAV	Homovanillic acid
HNE	4-hydroxy-2- noneal
HO*	Hydroxyl radical
HOO*	Hydroperoxyl radical
HRP	Horseradish peroxidase
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
iNos	Inducible nitric oxide synthase
IP	Intraperitoneal

List Of Abbreviations

IV	Intravenous
Kg	Kilogram
LBs	Lewy bodies
L/C	Levodopa/ Carbidopa
L-dopa	Levodopa
LPS	Lipopolysaccharide
LPO	Lipid peroxidation
LRRAK2	Leucine-rich repeat serine /threonine- protein kinase 2
MAO	Monoamine oxidase
MAOIs	Monoamine oxidase inhibitors
MAPK	Mitogen _activation protein kinas
MC-1	Mitochondrial complex-1
MDA	Malondialdehyde
Mg	Milligram
MPTP	1-methyl-4-phenyl tetrahydropyridine
MTT	(3-(4,5- Dimethylthiazole-2-yl)-2,5- diphenyl-2H-tetrazolium bromide)
NF-κB	Nuclear factor kappa B
NMDA	N-Methyl-D-aspartic acid
NMS	Non-Motor Symptoms
NO	Nitric oxide
6-OHDA	6-hydroxydopamine
O2.	Molecular oxygen

List Of Abbreviations

O.-2	Superoxide radical
OH·	Hydroxyl radical
OS	Oxidative stress
PBS	Phosphate buffer solution
PD	Parkinson disease
PGE2	Prostaglandin E2
PINK1	Serine/threonine-protein kinase
UPS	Ubiqutin proteasome system
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ROT	Rotenone
RPMT-1640	Roswell Park Memorial Institute-1640
SEM	Standard error of the mean
SN	substantia nigra
SNpc	substantia nigra pars compacta
SOD	superoxide dismutase
TH	Tyrosine hydroxylase
TNF-α	Tumor necrotic factor-α
TAOC	Total antioxidant capacity

1.1.Introduction

Parkinson's disease (PD) is a neurodegenerative disorder caused by the progressive loss of dopamine nerves in the substantia nigra (SN) of the midbrain (Rabiei and Solat, 2019).A relative increase of acetylcholine is caused by the loss of dopamine-secreting neurons, excessive acetylcholine is thought to be responsible for the motor impairments observed in PD. Neuroinflammation, mitochondrial dysfunction, and apoptosis are the main factors contributing to PD neurodegeneration (Abdelkader et al. 2020).

The Global Burden of Disease Study estimates that the number of PD cases will double from about 7 million in 2015 to about 13 million in 2040 ,suggesting a potential 'PD Pandemic' (Jankovic and Tan, 2020). The prevalence of PD is about 1% in people aged over 65 years. It begins between 40 and 70 years of age and is very rare for those under 20 years (Rabiei and Solat, 2019).

Parkinson's disease is multisystemic neurodegenerative condition that combines motor and non-motor symptom (NMS). Motor symptoms include movement and physical activity: tremors, stiffness, slowness, and imbalance. NMS, such as the gastrointestinal and genitourinary systems, are heterogeneous (Armstrong and Okun, 2020).

Several hypotheses have been raised for the death of dopaminergic cells in the SN par compacta (SNpc), such as mitochondrial complex defect associated with the electron transport chain, iron and protein accumulation, inflammatory immune responses along with environmental factors such as physical trauma and infection and increased formation of free radicals. Reactive oxygen species (ROS) and oxidative stress are currently thought to play a role in the pathogenesis of PD,

which results in damage to SNpc, particularly changes in iron control in the brain, mitochondrial dysfunction, changes to one's antioxidant regimen, especially reducing superoxide dismutase (SOD) and glutathione (GSH), as well as oxidative damage to lipids, proteins, and DNA (Rabiei and Solat, 2019). Reactive oxygen species-induced molecules released from damaged dopaminergic neurons can evoke microglial activation and pro-inflammation. Activated microglia released free radicals such as nitric oxide and superoxide, as well as pro-inflammatory cytokines including interleukin-1 Beta (IL-1β) and tumor necrosis factor-α (TNF-α), and drive proteases progressive neuronal damage (Saad et al. 2017).

Exposure to toxic chemicals and head injury may increase the risk of developing PD (Simon, Tanner, and Brundin 2020).

An environmental neurotoxin such as rotenone (ROT), generated from plants, is linked to the etiology of PD. ROT disrupts mitochondrial function by inhibiting mitochondrial complex-I (MC-1), which leads to progression of neurodegeneration and neuroinflammation in PD (Javed and Meeran, 2020). ROT caused a reduction in dopamine and tyrosine hydroxylase (TH) expression in neurons. The reduction of TH expression caused a reduction of dopamine in striatum content as the enzyme TH limits the rate of DA production (Abdelkader et al. 2020).

In general, the usage of natural products, including herbal medicine, can be attributed to the negative effects of chemical medications (Rabiei and Solat, 2019). The neuroprotective effect of medicinal plant extracts and phytochemicals in the reduction sign of PD due to antioxidant and anti-inflammatory features has been underlined in several studies (Khazdair and Kianmehr, 2021). The herb *B.officinalis* is a member of the Boraginaceae family (Parikh et al. 2019). Agents

with antioxidant and anti-inflammatory features that play a crucial role in preserving neurons and alleviating PD symptoms (Khazdair and Kianmehr, 2021). The edible flowers are an interesting source of bioactive chemicals with beneficial health-promoting qualities (Moliner et al. 2022).

1.2.Aims of the Study

This study aims to evaluate the neuroprotective activity of *B.officinalis* extract (BOE) in vitro and in vivo through the following objectives:

In vitro

(1) Examine the cytotoxicity of *B.officinalis* flowers extract (BOE) on SH-SY5Y cells.

(2) Study the immunological and biochemical effects of (BOE) on the levels of IL-1β, MDA and TAOC and identify the doses that effectively decrease the levels of IL-1β and MDA in SH-SY5Y cells .

In vivo

(1) Study the immunological and biochemical effects of (BOE) on the levels of IL-1β, MDA and TAOC and identify the doses that effectively decrease the levels of IL-1β and MDA in rats .

(2) Identify the effective doses in attenuating the Parkinson’s disease signs.

1.3. Parkinson's Disease

Parkinson's disease (PD) is the second-most prevalent neurodegenerative disease. Presently, there are more than 5 million PD patients in the world. The primary characteristics of PD are the loss of dopaminergic (DA-ergic) neurons in the (SNpc) and the absence of dopamine in the striatum (Han et al. 2022).

Parkinson's disease is a multisystemic neurodegenerative condition that combines motor and non-motor symptoms (NMS). NMS has been researched recently and is suggested in more than 90% of PD patients. The typical NMS might manifest at any stage of the PD and include dementia, anxiety, and pain. Cognitive impairment is a frequent NMS in PD patients (Vastegani et al. 2023).

Progressive degeneration caused by PD can be delayed but not completely stopped by treatment. Tremors in the upper and lower extremities, sluggish movements, and loss of balance are all signs, it is due to the neurodegeneration of the DA-ergic nigrostriatal pathway, which causes insufficient dopamine in the striatum (Al-Abbasi et al. 2022).

The neurotic features of PD include an uncommon accumulation of α-synuclein (α-syn), particularly across various brain regions in the residual DA-ergic neurons of the nigrostriatal pathway. Although (α-syn) aggregation intensively contributes to disease development, the mechanism that induces intraneuronal aggregation is still unknown (Issa et al. 2020).

Subtypes of PD consists of three groups:

1.Mild motor predominant: younger age at onset, mild motor and NMS, slow progression, good medication response.

2.Intermediate: intermediate age at onset and symptomatology, moderate-to-good response to medications.

3. Diffuse malignant: base line motor symptoms accompanied by rapid eye movement sleep behavior disorder, mild cognitive impairment, orthostatic hypotension, worse levodopa response, more prominent dopaminergic dysfunction and rapid progression (Armstrong and Okun, 2020) (Figure 1.1).

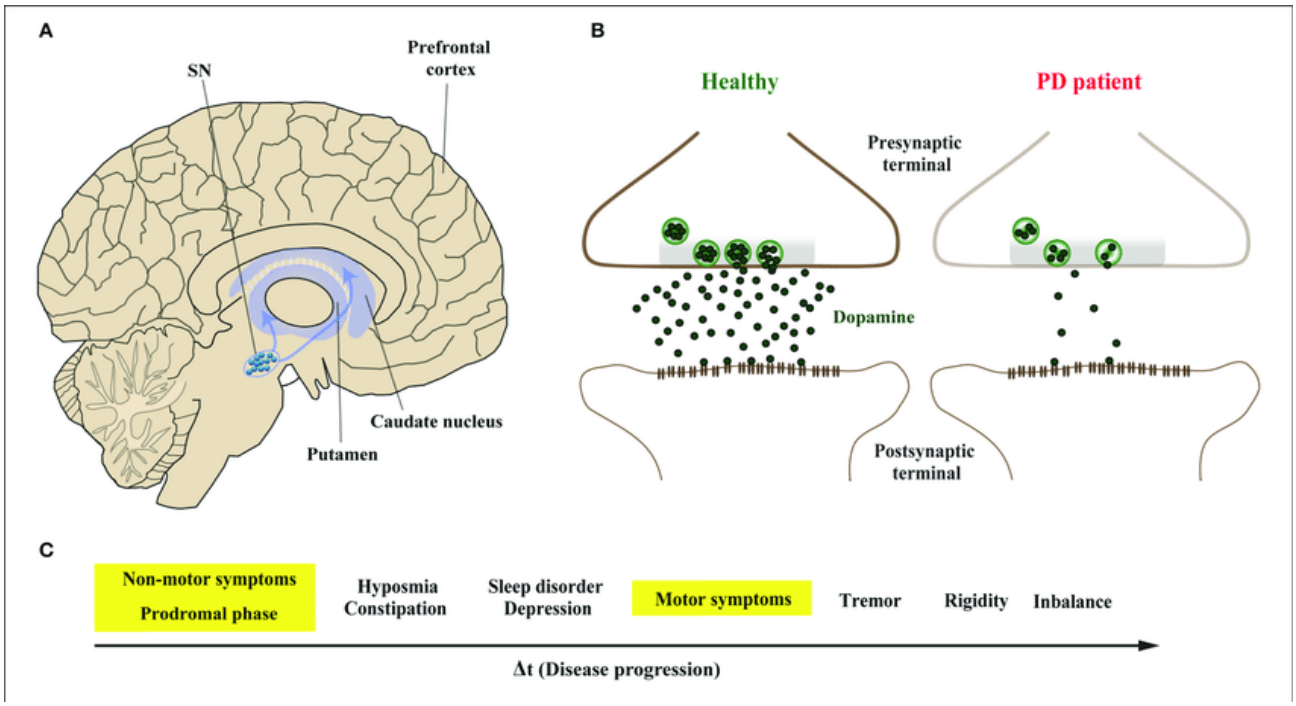

Figure 1.1: | Midbrain dopaminergic neurons are specifically vulnerable in Parkinson disease (Bridi and Hirth, 2018)**.**

1.4.Etiology of Parkinson's Disease

Parkinson's disease etiology is unknown (Rabiei and Solat, 2019).The prevalence appears to be higher in males than females (the range of the ratio is 1.3 to 2.0). However, smoking and caffeine use may impact incidence (Jankovic and Tan, 2020).

Idiopathic, environmental, and genetic variables all have a role in the etiology of PD. Over 95% of PD cases are idiopathic, including dopaminergic (DA-ergic) neuron atrophy due to the accumulation of Lewy bodies (LBs). LBs, abnormal aggregates of proteins that form in the substantial nigra (SN) and are thought to be the cause of neuronal atrophy, the existence of LBs causes the DA-ergic and non-dopaminergic neurons in the SN to degenerate, compromising motor control. Therefore, it is believed that idiopathic LBs aggregates are the primary factor responsible for the development of PD (Prete and Ouanounou, 2021).

It has been established that genetic variables play a significant role in the onset of PD. No genetic component is identified in 23% of instances (Rabiei *et al.,* 2019). Mutations in gene (PRKN) induce early-stage PD with autosomal recessive inheritance. In contrast, mutations in gene (LRRK2), one of the causal genetic alternatives for PD, account for a percentage of autosomal dominantly inherited PD cases (Chang and Chen, 2020).

Numerous risk factors have been identified, including type 2 diabetes, exposure to pesticides and heavy metals, rural life, agricultural work, head injury, history of melanoma, excessive dairy consumption (Jankovic and Tan, 2020).

The biological connection between environmental, genetic, and epigenetic factors depicted in (Figure 1.2).

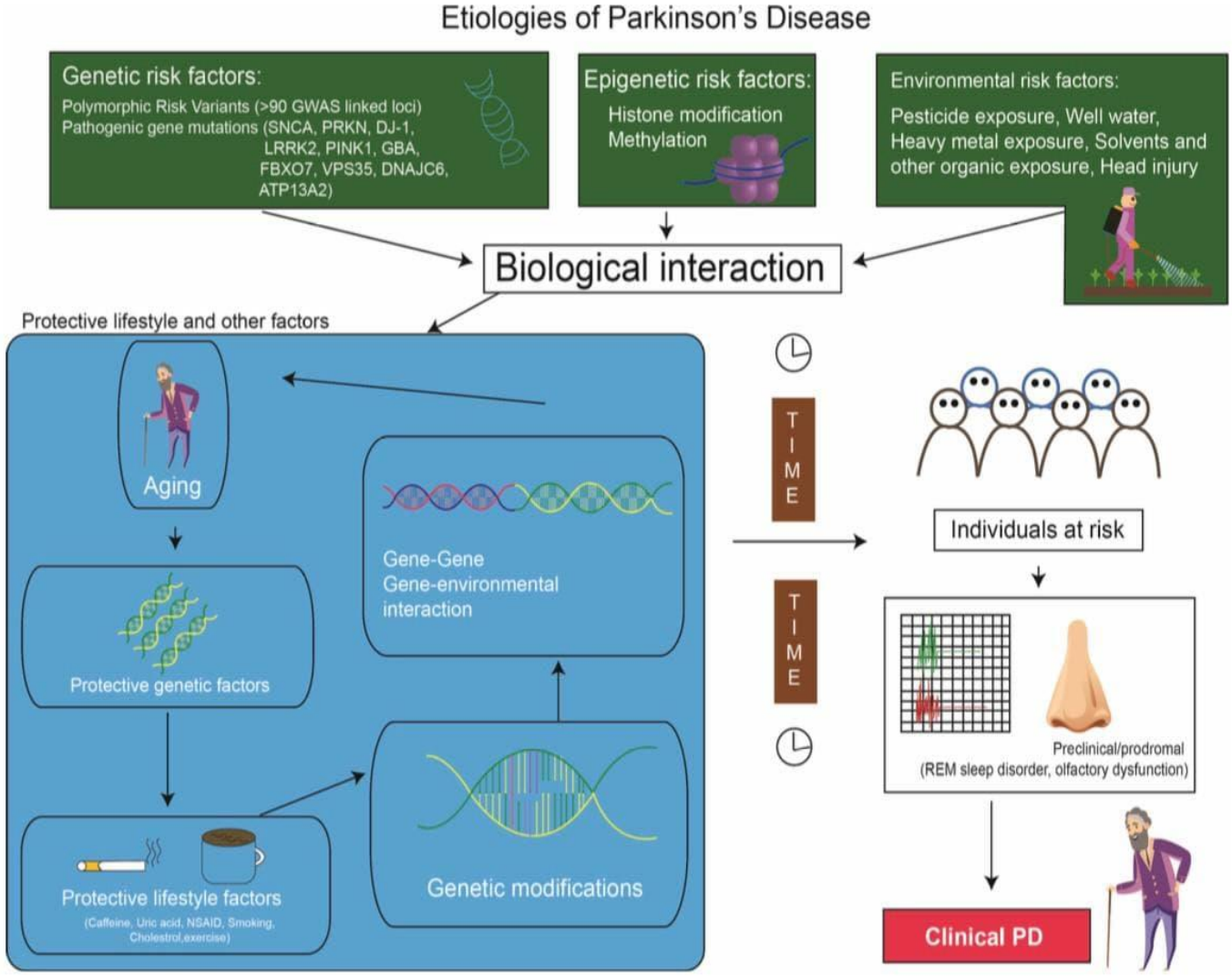

Figure1.2:Parkinson's disease Etiologies:The biological connection between environmental, genetic, and epigenetic factors (Jankovic and Tan, 2020)**.**

1.5. Pathophysiologic Mechanism of Parkinson's disease

The Braak hypothesis is a widely used theory to explain the neuropathological development of Parkinson's disease.

According to this hypothesis, PD in stages 1 and 2 begin in the medulla and olfactory bulb. Early pathologies, such as reduction in smell and rapid eye movement sleep behavior disorder (where people lose their typical rapid eye movement sleep paralysis and physically play out their dreams while they are asleep), are linked to the start of movement disorders. In stages 3 and 4, the SNpc as well as other midbrain and basal forebrain areas, are affected by the disorder. It is linked to classic motor signs of PD. At this point, PD is often diagnosed. In advance, PD progresses to the cerebral cortices with the onset of cognitive impairment and hallucinations (Armstrong and Okun, 2020) (Figure 1.3).

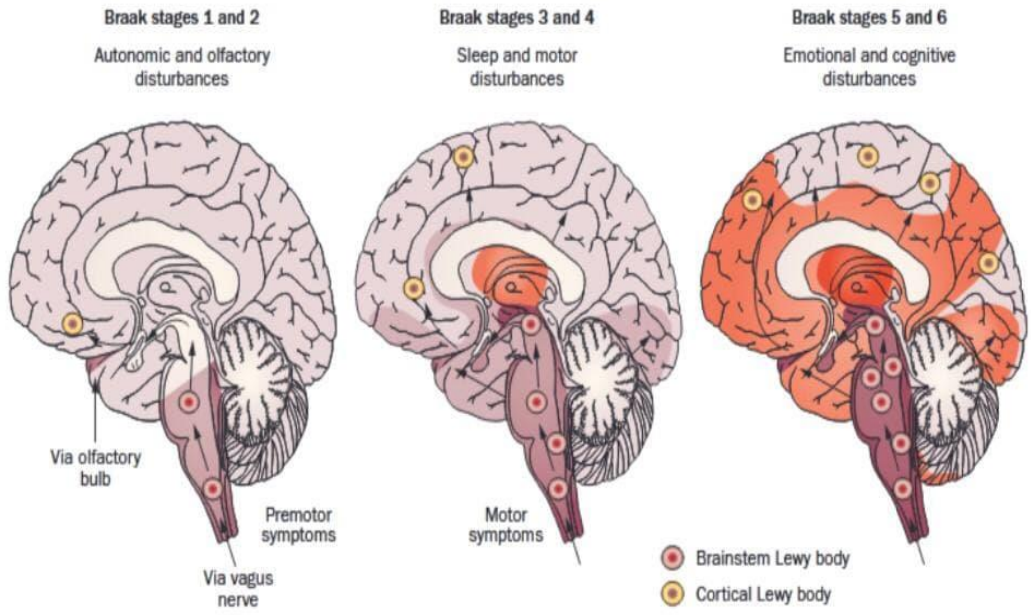

Figure 1.3: Braak staging. (Richard *et al.*, 2012)

1.5.1.Oxidative Stress

Oxidative stress plays an important role in the degeneration of DA-ergic neurons in PD leading to disruption of physiologic maintenance of the redox potential in neurons by interfering with several biological processes, ultimately leading to cell death (Dias, Junn, and Mouradian 2013). There are two basic processes that cause oxidative stress, the first is enzymes tyrosine hydroxylase and monoamine oxidase of ROS pathway, which are responsible to make DA-ergic neurons prone to oxidative damage, the second mechanism is the fenton reaction, which occurs in nigral DA-ergic neurons due to the presence of iron, which enhances oxidative stress by producing superoxide radicals and hydrogen peroxide (H2O2) (Khan and Athar Ali, 2019). Compared to other organs, the brain uses high amount of oxygen, causing it more vulnerable to free radical damage. Free radicals are extremely unstable molecules that can easily pass through biomolecules, including lipids, proteins, nucleic acids, and cell membranes. Reactive nitrogen species (RNS) and reactive oxygen species (ROS), two types of free radicals, are the primary catalysts for oxidative stress and an imbalance in the antioxidant capacity of cells. ROS are produced by human bodies when antioxidants cannot control this generation. This condition is known as oxidative stress and typically results in DNA, lipids, and protein damage to cells. Oxidative stress has a significant role in the etiology of PD, according to several experimental research on dopamine metabolism, lipid peroxidation (LPO), and glutathione depletion (Yin et al. 2021). The substantia nigra expresses GSH, an essential "scavenger" of ROS like free radicals, peroxides, and LPO in cells, at a comparatively low level compared to other brain regions, including the cortex, hippocampus, and cerebellum. The loss of GSH may not be the main cause of the

damage to the nigral neurons, it may also make them more vulnerable to free radical exposure (Rabiei and Solat, 2019).

Specific antioxidant defense mechanisms have been found in the body, which involve enzymatic and non-enzymatic pathways to destroy harmful free radicals. The mechanism of enzymatic defense involves three main enzymes superoxide dismutase (SOD), catalase (CAT) and Glutathione Peroxidase (GPX). These enzymes induce different reactions, and their target is to reduce the exaggeratedly produced ROS or to maintain a balance between the production and destruction of ROS and non - enzymatic like Vitamin-C (Parikh et al. 2019).

Mitochondrial damage and oxidative stress result in the formation of hydroxyl radical (•OH), the most potent and destructive oxidant via the Fenton reaction (Tarafdar and Pula, 2018).

In the Fenton reaction, H2O2 accepts an electron from ferrous iron (Fe2+) to produce ferric iron (Fe3+) and the hydroxyl radical (HO*). Fe3 has reduced back to Fe2+ by another molecule of H2O2, forming a hydroperoxyl radical (HOO*). The radicals damage cell membranes and mitochondria and also provoke apoptotic signaling (DiPro *et al.,* 2020) (Figure 1.4).

The pathway of ROS generation and induction of dopamine neurons death depicted below in Figure 1.5.

$$O_2^{\bullet-} + Fe^{3+} \_\_\_\_\_ Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \_\_\_\_\_ Fe^{3+} + OH^- + OH^{\bullet}$$

$$O_2^{\bullet} + H_2O_2 \_\_\_\_\_ OH^- + OH^{\bullet} + O_2$$

Figure 1.4: Fenton reaction

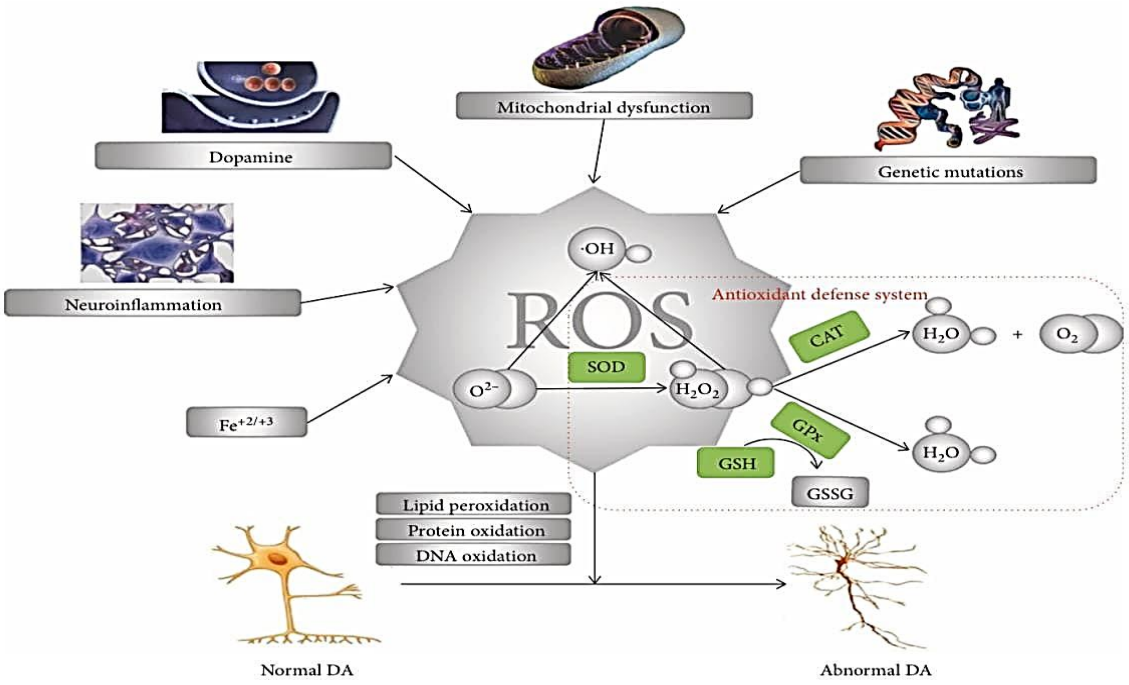

Figure 1.5: Schematic pathway of ROS generation and induction of DA neurons death. (Weng et al. 2018)**.**

1.5.2. Mitochondrial Dysfunction

Mitochondria are cellular energy producers and maintain homeostasis in cells. Their impairment causes oxidative stress and triggers death signal pathways leading to neurodegeneration (Witucki et al. 2022).

The prognosis for both familial and idiopathic PD is thought to be heavily influenced by the loss of mitochondrial activity. An absence of the mitochondrial complex-1 (MC-I) was found in earlier autopsy investigations in the substantial nigra (SN) of PD brains, which revealed one of the most important direct relationships between mitochondrial dysfunction and PD. Cell death and DA-ergic energy depletion are the results of MC-I dysfunction (Prasad and Hung, 2020).Complex I activity is inhibited by the accumulation of α-synuclein inside the inner mitochondrial membrane, which results in mitochondrial dysfunction and increased oxidative stress (Chang and Chen, 2020). Neurotoxin likes Rotenone triggers mitochondrial damage, resulting in decreased mitochondrial potential, cytochrome-C release, caspase cascade activation, and ultimately cell death. Additionally, resulting from the adverse effects of several PD-associated genes, specifically PINK1, Parkin, and DJ1(7,12,58), impaired mitophagy is one of the signs of mitochondrial dysfunction identified (Jankovic and Tan, 2020).

1.5.3. Protein Misfolding

The mechanism remains unavailable ,protein misfolding and aggregation are the most common molecular phenomena and causative factors for the pathogenesis of PD. For example, the protein of SNCA, PARK2, PINK1, DJ-1, and LRRK2 frequently misfold in the substantial nigra par compacta (SNpc) of the midbrain due to the mutations in their gene (Yin et al. 2021).

α-Synuclein (α-syn), a 140 amino acid small protein located at presynaptic nerve terminals that regulates the synaptic activity and neurotransmitter release, was genetically and neuropathologically linked to PD. Higher levels of (α-Syn) in plasma and serum explained a significant correlation between the severity of motor impairment and cognitive decline. (α-syn) undergoes extensive posttranslational modifications, including phosphorylation and conformational transformations that cause the aggregate formation and neuronal toxicity (Tönges et al. 2022).

Numerous mechanisms have been proposed for (α-syn) abnormal structural alterations and aggregation, including phosphorylation of serine-129, ubiquitination, and C-terminal truncation. As a result, various (α-syn), including unfolded monomers, soluble oligomers, and high molecular weight insoluble fibrils, accumulate in the PD brain (Prasad and Hung, 2020).

1.5.4. Lipid Peroxidation and Parkinson's Disease

Lipid peroxidation (LPO) is a fundamental constituent of oxidative stress and free radical production . In particular, reactive oxygen species (ROS) are able to attack polyunsaturated fatty acids (PUFAs) of cellular membranes, leading to structural impairment of the membranes, eventually generating a group of α, β-unsaturated highly reactive aldehydes, among which 4-hydroxy-2-noneal (HNE), malondialdehyde (MDA) and acrolein being the most reactive. Consequently, these strong reactive aldehydes are greatly diffusive and capable of attacking or forming covalent linkages with farther cellular constituents. MDA is one of the most mutagenic LPO products, being capable of reacting with deoxyguanosine and deoxyadenosine in DNA, thus generating mutagenic DNA adducts. Acrolein has been associated with α-synuclein modification in dopaminergic neurons, ultimately

causing mitochondrial dysfunction. Under conditions characterized by high LPO, dopamine is oxidatively converted to o-quinone, which in turn initiate a series of spontaneous reactions. In this case, intramolecular cyclization and molecular interaction with specific targets leads to cytotoxic responses and changed cell functioning . Accordingly, HNE has been found to modify transport and probably loss of dopamine. Thus, HNE elevation, protein accumulation and dopamine loss eventually affect learning and physical capabilities of PD patients (Taso et al. 2019).

1.5.5. Neuroinflammation

Neuroinflammation plays an important role in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).The neuroimmune response plays an essential role in the repair mechanism after an injury to the brain. Activation of the central and peripheral immune system after neurotrauma contributes to the tissue repair mechanisms as well as pathological state that can persist over a long period of time as a chronic reaction (Kempuraj et al. 2021). The inflammatory responses to PD involve the invasion of peripheral immune cells and the activation of glial cells (Prasad and Hung, 2020).

Activated microglial cells might contribute to DA-ergic cell death by releasing cytotoxic inflammatory compounds such as proinflammatory cytokines (TNF-α, IL-1ß, and interferon γ). Among these cytokines, TNF-α might have a direct damaging effect on dopaminergic neurons by activating an intracellular death pathway coupled with TNF receptor 1 expressed on the cell surface of these neurons. Pathways transduced by activation of TNF receptor 1 are linked to the induced expression of COX-2 within dopaminergic neurons. However, these

cytokines might also stimulate the expression of iNOS within microglial and possibly astrocytic cells through the expression and activation of the low-affinity receptor of immunoglobulin E (CD23). This process might lead to the production of toxic amounts of NO free radicals. In turn, these free radicals could potentiate the expression and release of TNF-α by adjacent microglial cells, thereby amplifying further the inflammatory reaction (Hirsch and Hunot, 2009).

1.5.5.1. Mediator During Neuroinflammation

Nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β(IL-1β), cathepsin-B, and interleukin-8 (IL-8) are inflammatory mediators produced due to microglial activation and have been shown to induce apoptosis. In addition to being identified extremely high levels in the substantial nigra par compacta (SNpc) tissue of postmortem PD patients as well as in the cerebral spinal fluid (CSF) of PD patients, a large number of these inflammatory mediators (NO, IL-1β, IL-6, TNF-α, and prostaglandin-E2 (PGE2) are also occasionally produced by microglial cells only when NF-κB is activated. Dopamine neuronal loss caused by immune system damage or dopamine neurotoxins causes microglial cells to become activated (Borowy, Chwil, and Kapłan 2017). Inhibitors of NF-κB can be used to reduce reactive microgliosis, which is the primary indicator of chronic neuroinflammation in PD (Singh et al. 2020).

Multiple studies support the hypothesis that COX-2 potentiates the cytotoxic effects not only through the ROS generated during conversion of prostaglandins-G to prostaglandins-H but also by producing pro-inflammatory prostaglandins that leads microglial activation. Besides COX-2, iNOS, another enzyme participate in dopaminergic neurodegeneration by production of iNOS involving activated

microglia which results in the increased formation of NO and subsequently deleterious effect on DNA and proteins (Javed and Meeran, 2020).

1.5.5.2. Transcription Factor NF- κB in Parkinson's Disease

Nuclear Factor-κB (NF-κB), an key factor known to be capable of expressing pro-inflammatory cytokines in neurons in PD (Khazdair and Kianmehr, 2021). NF-κB was found to show expression in various cells and tissues, such as microglia, neurons, and astrocytes which play an important role in activation and regulation of inflammatory intermediates during inflammation. Both canonical and non-canonical NF-κB pathways are involved in the regulation of the stimulated cells. The canonical pathway is the most extensively studied pathway of NF-κB activation triggered in response to pro-inflammatory molecules such as TNF, IL-1, and T-cell receptor or B cell receptor. The non-canonical NF-κB pathway is activated in response to various stimuli including members of TNF receptor super family such as B cell activating factor (BAF), receptor activator of NF-κB (RANK) , lymphotoxin β (LTβ) receptor, and CD40 (Singh et al. 2020).

NF-κB may be responsible for activating numerous inflammatory cytokines and enzymes, including Inducible nitric oxide synthase (iNOS) and COX, as well as the activation of other pathways linked to oxidative stress. NF-κB activation is also regulated by the mitogen-activated kinase pathway (MAPK). The MAPK pathway regulates many cell functions, including mitosis, metabolism, and apoptosis (Parikh et al. 2019).

1.5.6. Instability of Metal Ions Homeostasis

In physiological conditions, ions (in particular calcium and iron) have been explicitly demonstrated to be implicated in various vital biological processes including DNA biosynthesis, myelin sheath and neurotransmitters, mitochondrial respiration, and brain development and metabolism. The accumulation of iron in the SNpc and reticulata of PD patients has been frequently observed, which also increases with disease severity (Yin et al. 2021). Pro-oxidant interactions between iron and dopamine are provided to be enhanced in ageing substantial nigra par compacta (SNpc) due to the accumulation of iron in the same brain region. Iron accumulation is considerably enhanced compared with healthy ageing, in the postmortem SNpc, iron levels are increased twofold compared with age-matched controls (Trist, Hare, and Double 2019). Iron chelators may be used to treat Parkinson disease due to the iron deposition in substantial nigra and its resulted to oxidative stress synthesis. Desferrioxamine, a common iron chelator, normalizes hydroxyl radical and lipid peroxidation (LPO) levels while inhibiting iron accumulation (Chang and Chen, 2020).

The cytosolic Ca2+ in SNpc DA neurons is mainly responsible for three complementary functions: (1) helps maintain the slow tonic spiking in these neurons, even though it is not required for pacemaking (2) positively modulates the expression and activity of enzymes involved in DA synthesis, ensuring a match between the supply and demand of the neurotransmitter, and (3) stimulates oxidative phosphorylation and ATP production (Yin et al. 2021).

ATP-dependent pumps in mitochondria are necessary for regulating intracellular Ca2+, which increases ROS generation (Chang and Chen, 2020).

1.5.7. Protein Synthesis and Degradation in Parkinson's Disease

There are two central protein clearance systems within cells responsible for the removal of dysfunctional proteins: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway. The UPS is primarily responsible for breaking down abnormal proteins, and it does so by "tagging" them with ubiquitin and transporting them to the proteasome for degradation. The autophagy-lysosome pathway is divided into three constituents: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). In macroautophagy, intracellular components, including cytosolic proteins are engulfed by the autophagosome, which then fuses with the lysosome, leading to the breakdown of its contents. On the other hand, in microautophagy, the lysosome alone engulfs and destroys cytoplasmic components. CMA is a more selective process, whereby molecular chaperones target specific proteins and transport them to the lysosome for degradation (Stoker and Greenland, 2018).

1.5.8. Strategies of Parkinson's Disease Neuroprotective Targets

There is plenty of opportunity for neuroprotection in PD, considering that the prodromal stage may last from 5 to 20 years. Neuroprotective strategies, probably the greatest unmet need in PD therapy, include: (i) buffeting compensatory mechanisms, (ii) salvaging dying neurons or "neurorescue," and (iii) replacing degenerating neurons via cell-based therapy or neurorestoration. Although several putative agents have been investigated, none have yielded significant promise (Garg and Desai, 2021).

Development of neuroprotective strategies has been challenging, partly because of lack of reliable and sensitive biomarkers of progression and yet incomplete

understanding of the pathogenesis of the disease. One of the most exciting developments of potential neuroprotective or disease modifying therapies is the use of (α-syn) monoclonal antibodies to minimize accumulation and spread of aggregated, toxic α-synuclein (Jankovic and Tan, 2020).

1.6. Diagnosis of Parkinson's Disease

Parkinson disease is diagnosed using a history and physical examination. History can include prodromal features (eg, rapid eye movement sleep behavior disorder, hyposmia, constipation), characteristic movement difficulty (eg, tremor, stiffness, slowness), and psychological or cognitive problems (eg, cognitive decline, depression, anxiety). Examination typically demonstrates bradykinesia with tremor, rigidity, or both (Armstrong and Okun, 2020).

1.7. Pharmacological Treatment of Parkinson's Disease

1.7.1. Levodopa (L-dopa)

Dopamine agonists are used as the first choice in treating Parkinson’s disease (PD) and remain symptomatic. Treatments can slow the progression of PD, and most of them only relieve symptoms (Okkay and Ferah Okkay, 2022).

Levodopa (L-dopa) is the most efficient treatment for motor symptoms in the early stages, but it is not considered a treatment for PD, the rigidity response better and the tremor may be reduced slightly. However, L-dopa is ineffective in preventing loss of neurons, non-motor symptoms, or Lewy bodies (Balakrishnan *et al.* 2021).

Patients typically begin with L-dopa at a low dose. Most individuals need a dose of between 150 and 1000 mg per day, divided over several doses, larger doses result in a high chance of major side effects, prolonged use can cause serious motor problems, including dyskinesia and severe on-off motor fluctuations that must be discontinued (Stoker and Greenland, 2018).

1.7.1.1. Biochemistry of L-Dopa

Dopamine's precursor, L-Dopa, is an aromatic amino acid with a molecular formula of $C_9H_{11}NO_4$. It is a prodrug converted to dopamine by DOPA decarboxylase and can cross the blood brain barrier (BBB). It is the most effective and widely used treatment for PD. It is typical to take (L/C), an inhibitor of the plasma enzyme (L-amino acid decarboxylase) that indirectly metabolizes L-Dopa. Several metabolic pathways convert L-Dopa to dopamine, then turn to sulfated or glucuronidated metabolites, epinephrine E, or Homovanillic acid (HAV). 3,4-dihydroxy phenylacetic acid (DOPAC)(13-47%) and (HAV) (23-39 %) are the main metabolites (Ncbi, 2019) **(**Figure 1.6).

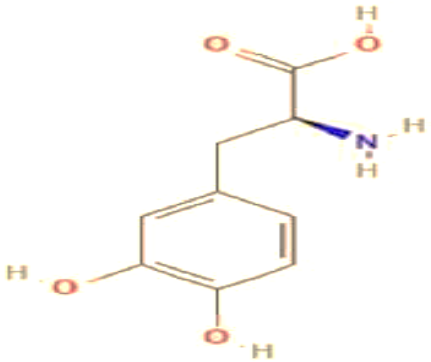

Figure1.6: Chemical structure of Levodopa

1.7.1.2. Pharmacokinetics of Levodopa

Levodopa (L-dopa) is quickly absorbed from the small intestine. However, its absorption is influenced by stomach pH and gastric emptying. Eating delays the appearance of L-dopa in plasma. The half-life of plasma is typically between 1-3 hours, and the peak plasma concentrations typically occur one to two hours after an oral dose. Only 1-3% of L-dopa that is administered makes it into the brain intact, the remainder is metabolized outside the brain, primarily via decarboxylation of dopamine, which cannot cross the blood-brain barrier. Consequently, L-dopa should be given in large amounts when used alone . Levodopa's duration of action may be prolonged by adding amantadine extended-release formulations or dopamine agonists or blocking DA metabolism using (MAOIs) or (COMTIs) (Jankovic and Tan, 2020) (Figure 1.7).

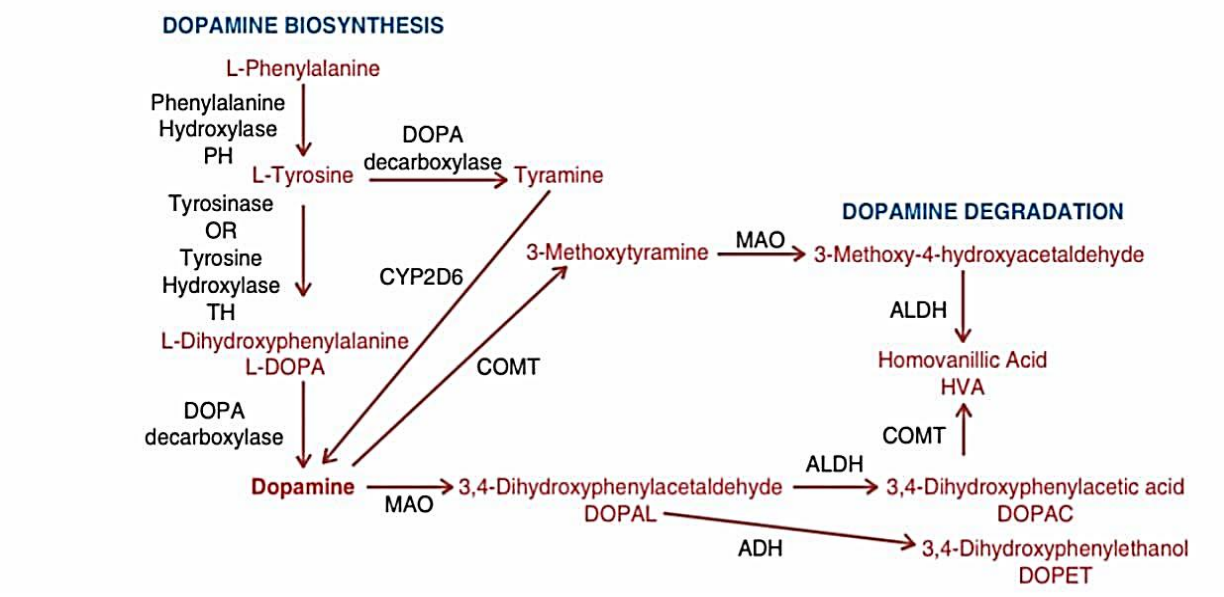

Figure 1.7: Dopamine's metabolic pathway: synthesis and clearance (Stoker and Greenland, 2018)

1.7.1.3. Form of Levodopa/ Carbidopa

Levodopa (L-dopa) exists in various formulations created or is currently being developed to distribute the drug desirably while avoiding or preventing problems. Although the reduction in a day 'off' time of about 1 hour is minor, like other supplementary oral medications, extended-release L-dopa/ Carbidopa (L/C) is helpful in patients who still experience vasomotor irregularities despite a greater dose of L-dopa (Jankovic and Tan, 2020).

There are different forms of (L/C) include: Immediate-release (IR) tablets, Disintegrating tablets (Parcopa) , Controlled-release (CR) tablets and Extended-release (ER) capsule (Rytary).

In 2018, the US Food and Drug Administration (FDA) approved an inhaled formulation of L-Dopa as an additional therapy to (L/C) for the treatment of PD , Inhaled L-dopa bypasses the intestinal absorption and hepatic metabolism of oral levodopa, and it is available in the form of dry powder (Gandhi and Saddabadi ,2021).

1.7.1.4. Response Fluctuations and Dyskinesias

For patients who have been on L-dopa medication for over ten years, dyskinesia can happen in up to 80% of cases. Although L-dopa dyskinesias' characteristics vary from patient to patient. As treatment progresses, specific variations in the clinical response to L-dopa happen more frequently. The timing of L-dopa delivery (end-of-dose akinesia or wearing-off reactions) may factor in certain patients' variations (Aminoff, 2018).

The term "on and off phenomenon" describes how people with severe Parkinson's disease may experience sudden changes in their ability to move. When the patient is in the "on" condition, motor symptoms are generally under control. However, the levodopa's effects quickly wear off, leaving him in the "off" state, where he exhibits severe motor symptoms of Parkinson's disease. These fluctuations can be particularly troublesome and severely restrict function (Stoker and Greenland, 2018).

1.7.2. Dopamine Receptor Agonists

Dopamine receptor agonists such as (Bromocriptine, Pergolide, Pramipexole, Ropinirole, Rotigotine,Apomorphine). All dopamine agonists activate DA receptors (D2). Treatment with dopamine agonists provides many advantages over L-dopa, because of enzymatic conversion of DA agonist is not necessary for their activity, they produce a direct stimulation to DA receptors, they have a longer half-life in comparison with L-dopa, they possess possible neuroprotective effects and the most important advantage is confirmed decreased occurrence of motor complications compared to L-dopa (El Sayed et al. 2018).

1.7.3. Monoamine Oxidase Inhibitor (MAOI)

Although selegiline and rasagiline are most frequently used in early, mild PD, these MAOIs are also effective in patients with moderately advanced PD with levodopa- related motor complications. Another MAOI, safinamide, administered once daily (50–100 mg/day), has been found to increase mean on time without troublesome dyskinesia and reduce daily and morning off times (Jankovic and Tan, 2020)

1.7.4. Catechol -O-Methyltransferase Inhibitors (COMT inhibitors)

Catechol -O-Methyltransferase Inhibitors such as Tolcapone, Entacapone. COMT is an enzyme involved in the peripheral degradation of L-dopa. Approximately 99% of the orally administered dose of L-dopa does not reach the brain but, rather, is decarboxylated to dopamine (DA), which causes nausea and hypotension. Addition of Carbidopa decreases the formation of DA but increases the fraction of L-dopa that is methylated by COMT. COMT inhibitors block the peripheral conversion of L-dopa to 3-O-methyl dopa, augmenting the bioavailability and the half-life of L-dopa, which is beneficial in patients with motor fluctuations (El Sayed et al. 2018).

1.7.5. Amantadine

Amantadine is an antiviral agent used for the prophylaxis and treatment of influenza A. By chance, it was found that amantadine has the ability to relieve early symptoms of PD as well as treatment of dyskinesia. Many mechanisms of action elucidate antiparkinsonian effects of amantadine where, it increases DA release and inhibits DA reuptake, blocks NMDA glutamate receptors. In addition, it has antimuscarinic activity (Armstrong and Okun, 2020).

1.7.6.Anticholinergics

Anticholinergics such as trihexyphenidyl and benztropine, antagonise the effects of acetylcholine at muscarinic receptors postsynaptic to striatal interneurons. They are predominantly used to reduce tremor and have no effect on bradykinesia (Jankovic and Tan, 2020).

1.8. Pharmacological Side Effects of Parkinson's Disease Drugs

1.Levodopa

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 (Kalinderi, Papaliagkas and Fidani 2019) .

2. Dopamine Agonists

The most common side effects of dopamine agonists include orthostatic hypotension, sleepiness, hallucinations and leg edema (Jankovic and Tan, 2020).

3.Monoamine Oxidase Inhibitors (MAOI)

These medications may increase the adverse effects of L-dopa, including nausea, dyskinesias, mental abnormalities, and trouble sleeping (Aminoff, 2018)

4.Catechol-O-Methyltransferase Inhibitors (COMTIs)

Tolcapone is associated with an uncommon, but potentially serious, risk of hepatotoxicity, and as such entacapone is generally preferred. Other side effects include sleepiness, nausea, loss of appetite, diarrhea, dizziness, orange urine

discoloration, hallucinations, abdominal pain, headaches, confusion, dry mouth, and chest pain (Stoker and Greenland, 2018).

5. Amantadine

Hallucinations, sadness, irritability, anxiety, excitation, sleeplessness, restlessness, and Confusion (Connolly and Lang, 2014).

6. Anticholinergics

Urinary retention, dry mouth, constipation, hallucinations, disorientation, and blurred vision (Jankovic and Tan, 2020).

1.9. *Borago.officinalis*

Taxonomical classification:

Kingdom : Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Lamiales

Family: Boraginaceae

Genus: Borago

L. Species: *Borago officinalis L*. (Ibrahim, Abdul, and Alshammaa 2023).

B.officinalis is widely diffused in Asia, Germany, France, Denmark, and the United Kingdom (Manthena et al. 2022).

B.officinalis is a plant coated with rough hairs, a thick stem ,it heightens to between 60 and 100 cm, simple leaves that smell of cucumber, and pink, light blue, and less commonly white flowers (Michalak and Zag, 2023).

B.officinalis flower development was classified into three stages:

- Stage 1 (termed tight bud stage) -bud flower closed, viewing some petal color at the tip of the bud end.
- Stage 2 (termed mature bud stage) -bud flower closed, viewing the color of petals in their entire surface.
- Stage 3 (termed fully open) -the flower completely opened, without symptoms of age (Fernandes et al. 2019) (Figure 1.8).

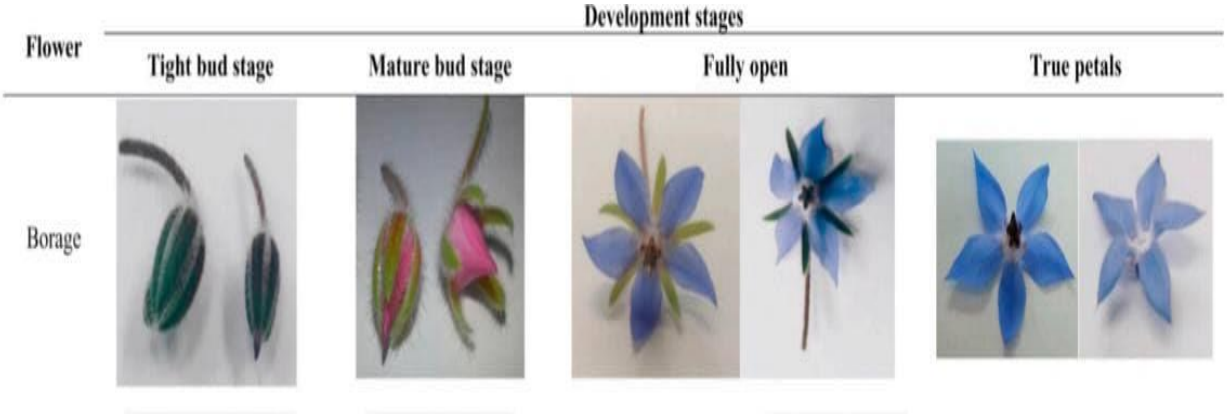

Figure 1.8: Petals and three flowering stages of borage (Fernandes *et al.*, 2019).

1.9.1.Phytochemical Composition

1. Borage Seed Oil

1. Polyunsaturated fatty acid: α-Linoleic acid (ALA) ,oleic + elaidic acids, γ-linolenic acid(GLA), palmitic acid ,stearic acid, cis-11-eicosenoic acid , and erucic acid.

2. Flavonoids .
3. Phenolic acids (Borowy and Kapłan, 2020) .

2. Borage Leaves

1. A few amounts of (pyrrolizidine alkaloids, intermedin, sopinin, yezan, and colin)
2. Fatty acids and Silicic acid.
3. Potassium, calcium, nitrate potassium.
4. Acetic, lactic and malic acid, cianozhens (Asadi-Samani and Bahmani, 2014).

3. Borage Flower

1. Phenolics (gallic acid, pyrogallol, salicylic acid, caffeic acid, rosmarinic acid , sinapic , Syringic and chlorogenic acids)
2. Fatty acids: including Alpha-linolenic acid (ALA) and Gamma-linolenic acid (GLA)
3. Flavonoids (myricetin, rutin, quercetin and kaempferol)
4. Isoflavonoid (daidzein) (Karimi et al. 2018).

Also, *B.officinalis* contain carbohydrates, tannins, saponins, mucous compounds, tocopherols, allantoin, mineral salts, vitamins, and volatile oil (Michalak and Zag, 2023).

1.9.2. Pharmacological activity of *Borago .officinalis*

In herbal medicine, the flower of borage is recognized as a sedative in a study involved formalin-induced pain. Hydroalcoholic extract administration acute and chronic pain and this may be related to its antioxidant activity that possibly

ameliorated the damaged cells stimulated by injection of formalin or suppressed the depolarization trigger in pain sensory neurons (Ibrahim et al. 2023).

Borage treats heart diseases, atopic dermatitis, diabetic neuropathy, rheumatoid arthritis, menopause-related symptoms, gastrointestinal and respiratory diseases due to its nutritional value. Borage flowers have sedative properties, and their leaves are used due to their anticonvulsant, bronchodilator, and vasodilator effects. The hydroalcoholic extract has topical anti-inflammatory, radical scavenging, and antioxidant effects (Akbar et al. 2020).

The cooking of leaves, flowers and stems of *B.Officinalis* were widely utilized in liver diseases, multiple sclerosis, abdominal pain and eczema (Michalak *et al.*, 2023).

B.officinalis flowers are a good source of carotenoids and polyunsaturated fatty acids (PUFAs), it also includes vitamin E and tocopherols, fat-soluble phenolic compounds, have antioxidant properties, and borage species contain high amounts of δ-tocopherols (Moliner et al. 2022) (Yaghmour et al. 2021).

Gamma-linolenic acid (GLA) is a precursor of prostaglandin E1 which includes the regulation of many metabolic roles, and multiple studies have shown that GLA can relieve the signs and symptoms of many chronic inflammatory diseases, including rheumatoid arthritis and atopic dermatitis (Borowy et al. 2020).

Phenolic acids play a significant role in destroying free radicals, reactive oxygen species (ROS) and inhibiting lipid peroxidation (LPO), serving as an anti-inflammatory and antioxidant agent. The main phenolic constituents are borage, rosmarinic acid, syringic acid, sinapic acid, rutin, and chlorogenic acid.

Rosmarinic has been indicated to diminish the production of nitric oxide(NO) and prostaglandin-E2 (PGE2), as well as Cyclooxygenase-2(COX-2) and Inducible nitric oxide synthase (iNOS) activity. Furthermore, Syringic acid has been proposed due to its anti-bacterial, anti-DNA oxidation, and anti-inflammatory properties. sinapic acid has been demonstrated to have anti-inflammatory and anticancer effects and revealed that sinapic acid has anti-inflammatory characteristics by preventing NF-κB activation in macrophages and iNOS, COX-2, and other proinflammatory cytokine production. Chlorogenic acids are phenolic acids with a hydroxyl group in the contiguous position and consist of esterification of cinnamic acids, ferulic acid, and quinic acid, chlorogenic acid has been indicated to have anti-inflammatory and antioxidant properties (Parikh et al. 2019).

1.10. Models of Parkinson's Disease

Disease animal models are essential for drug discovery. An ideal PD animal model would have all of the clinical and pathologic features of human PD (Prasad and Hung, 2020). These models achieve to some extent to reproduce the key features of PD, including motor defects, progressive loss of dopaminergic neurons in substantia nigra pars compacta, and the formation of Lewy bodies (Zeng, Geng, and Jia 2018). Difficulties associated with studying the early biological changes of PD arise as samples of the main organ affected, the brain, can only be investigated post-mortem. This limitation has led to the development of many animal models of PD, which can be broadly categorized into:

- toxin models induced by MPTP, 6-OHDA and rotenone.
- pharmacological models induced by reserpine and haloperidol (Johnson and Bobrovskaya, 2015).

- genetic models induced by genetic modifications of SNCA, UCH-L1, PINK1, DJ-1, LRRK2 (Zeng et al. 2018).

1.11. Neurotoxin Induced Models of Parkinson's Disease

Many models of PD are based on local or systemic delivery of neurotoxins causing the degeneration of produced dopaminergic neurons.

1. Rotenone

Rotenone (ROT) is a naturally occurring compound found in plants like Tephrosia and Lonchocarpus. ROT-treated animal models of Parkinson disease (PD) exhibit Lewy bodies in substantial nigra (SN) neurons and the degradation of these cells. One of the preferred experimental models for studies on neuroprotective treatments for PD uses animal or cell models exposed to ROT.ROT inhibits mitochondrial complex-1(MC-I) in neuronal cells, mediates α-synuclein aggregation, leaded to mitochondrial dysfunction and excessive generation of reactive oxygen species (ROS), and finally causes neuronal cell death (Han et al. 2022).

Rotenone treatment reduced levels of endogenous antioxidants, and increased oxidative and nitrative stress was observed in the animal's brains, which is dependent on oxidative stress and ROS generation as the mechanism behind ROT neurotoxicity. By inhibiting the respiratory chain of the mitochondrial complex-I enzyme, ROT prevents nicotinamide adenine dinucleotide (NADH) oxidation and causes neurotoxicity. Due to the great sensitivity of the dopaminergic neurons to oxidative damage to the SN, exposure to the ROT damages these neurons specifically and causes symptoms similar to PD (Al-Abbasi et al. 2022).

Rotenone is involved in the blood brain barrier (BBB) rupture caused by microglia activation and the neuroinflammatory response, which may cause memory loss and neuronal death (Vastegani et al. 2023).

Dopamine and tyrosine hydroxylase (TH) expression in striatal neurons decreased as a result of ROT. Because TH is a rate-limiting enzyme in dopamine synthesis, decreasing TH expression decreased dopamine content in the striatum, which is consistent with other results (Abdelkader et al. 2020) (Figure 1.9).

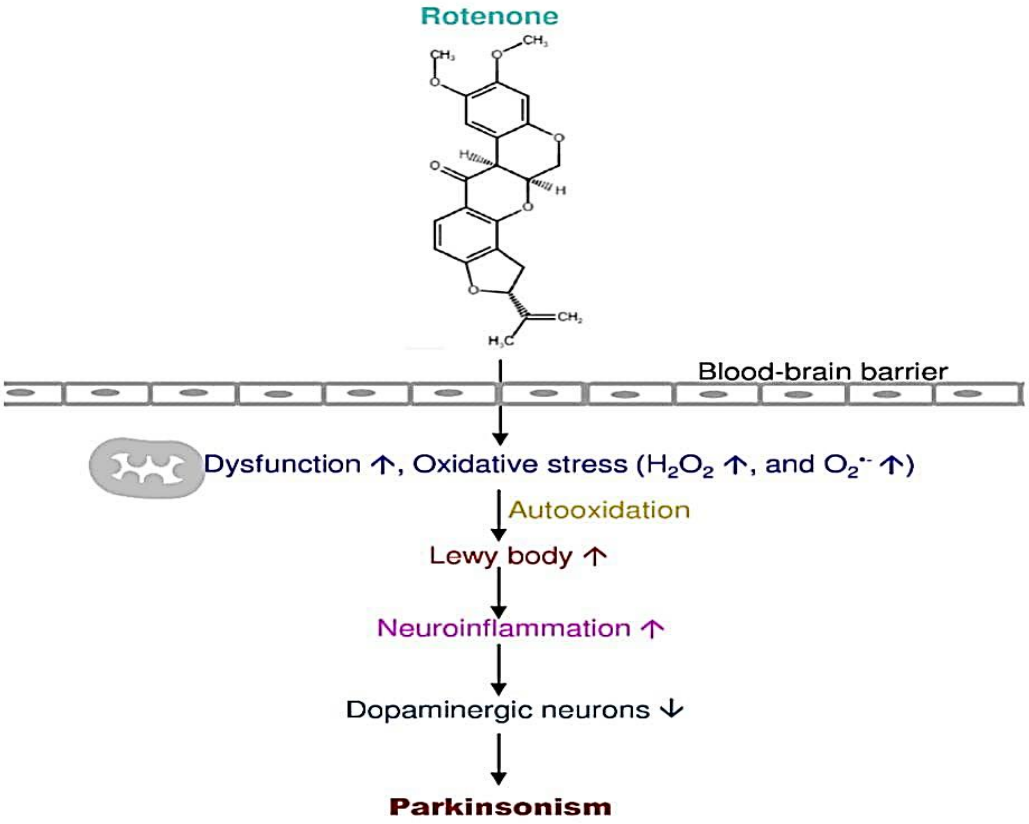

Figure 1.9:Mechanism of action of rotenone in Parkinson's disease progression (Prasad *et al*., 2020).

2. 6-hydroxydopamine

In order to investigate Parkinson disease, a fatal toxin that primarily harms the periphery and central nervous system (CNS), numerous 6-hydroxydopamine (6-OHDA) animal models have been used. Administration of 6-OHDA is unilaterally variable in the nigrostriatal pathway to generate neurodegeneration. 6-OHDA cannot penetrate the BBB, only direct delivery into the brain can cause central nervous system damage (Prasad and Hung, 2020).

3. Paraquat

It is a quaternary nitrogen herbicide that is extremely hazardous. According to numerous studies, paraquat produces significant lung damage followed by a phase of substantial compensatory fibrosis. As a result of its low cost and quick action, paraquat is a commonly used insecticide (Prasad and Hung, 2020).

4. 1-Methyl-4-phenyl tetrahydropyridine (MPTP)

Over the past thirty years, a rather basic molecule has significantly impacted understanding and PD therapy. MPTP is a byproduct of 1-methyl-4-phenyl-4-propionoxypyridine, a synthetic version of heroin. MPTP in the synthetic heroin causes selective destruction of dopaminergic neurons of the nigrostriatal pathway to produce PD symptoms in humans and other primates (Prasad and Hung, 2020).

Structures of dopamine and main neurotoxins used to reproduce features of PD in an animal model depicted in Figure 1.10.

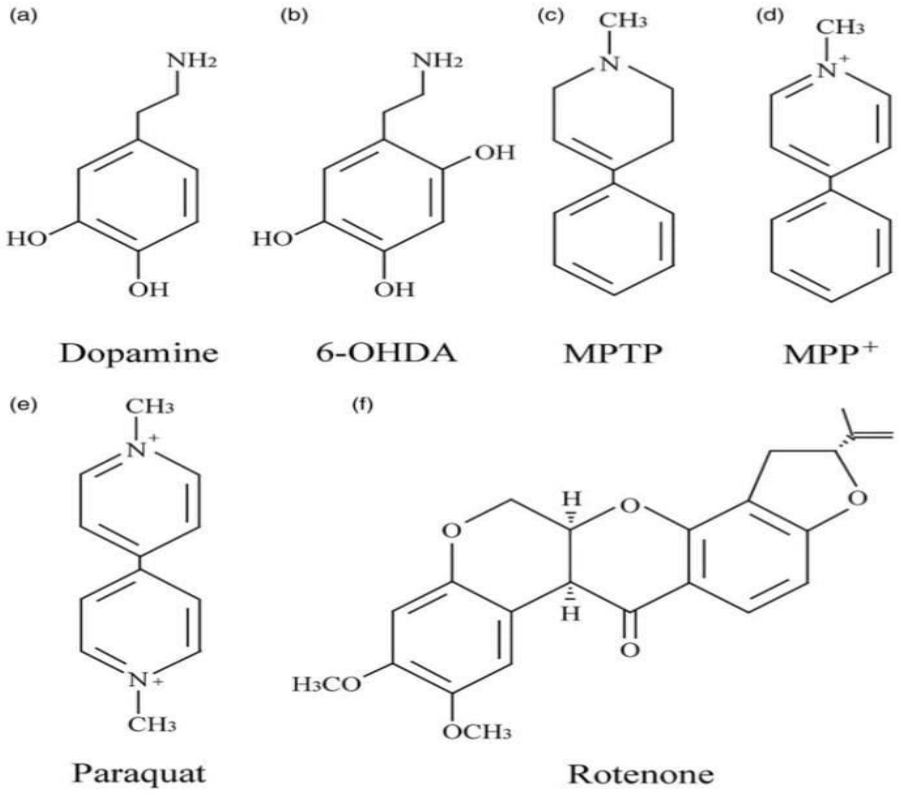

Figure 1.10:Structures of dopamine and main neurotoxins used to reproduce features of Parkinson's disease in an animal model (Zeng et al. 2018)**.**

1.12. Animal Cell Cultures

Animal cell culture is the process of human, animal, and insect cells developing in a supportive artificial environment. The artificial environment in which the cells are cultured invariably consists of a suitable vessel containing a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (O2, CO2), and regulates the physicochemical environment (pH, osmotic pressure, temperature) (Capes-Davis et al. 2010) **.**

Cells growing on explants or in dispersed cell suspensions (floating freely in culture fluid) are harvested using the most popular tissue culture technique. On a solid substrate, adherent monolayers of cells produced either through enzymatic treatment or mechanical means are grown.

There are three forms of cell culture.:

1. Precursor cell culture: These cells are committed to differentiating.
2. Differentiated cell culture: which is fully developed cells that cannot differentiate further
3. Stem cell culture: cells that have not undergone differentiation and can differentiate into any form of cell (Brier et al. 2020).

1.12.1. Advantages and Disadvantages of Cell Culture

The main advantage is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells. Cell cultures have a high control of the physicochemical environment (i.e., pH, temperature, osmotic pressure, oxygen, and carbon dioxide tension) which can be controlled very accurately, and the control of physiological conditions, which can be constantly examined. The disadvantages of cell culture are: highly skilled personnel, techniques must be performed using strict asepsis techniques because animal cells grow slower than many of the common contaminants (e.g., bacteria, viruses, and fungi). Additionally, animal cells may not survive when isolated and therefore are not capable of independent sustainable existence without providing a complex environment. One of the main limitations of cell culture is the expense and effort that has to be applied to obtain a relatively low amount of cells (Levy, Rojas-villarraga, and Levy 2000).

1.12.2. Primary Culture

Primary culture is turned to the stage of the culture where the cells have been removed from the tissue and multiplied under optimal conditions until they have filled the entire surface area (i.e., reached confluence). To provide greater space for further growth at this point, the cells must be sub-cultured (i.e., passaged) by moving them to a new vessel with a fresh growth medium (Chaudhary and Singh, 2019).

1.12.3. Cell Lines

The term cell line refers to the reproduction of culture after the first subculture. In simple terms, a cell line is created once the original culture has been sub-cultured. Multiple cell lineages with comparable or different characteristics can be found in a particular cell line. Cloning, physical cell separation, or other selection techniques can be used to choose a specific cell origin. Thus, a cell strain is a line of cells created through selection or cloning. Because cell strains have a restricted lifespan, they eventually die after some divisions (Chaudhary and Singh, 2019).

1.13. SH-SY5YCells Model for Parkinson's Disease

The SH-SY5Y human neuroblastoma cell line was one of the most used cell lines in neurosciences, either undifferentiated or differentiated into neuron-like cells. cells are an excellent model system for studying the effects of toxicity on both proliferating cells and differentiated cells. This neuroblastic cell line was created in the 1970s from a metastatic bone marrow biopsy from a four-year-old child with neuroblastoma. It is a thrice-cloned subline of the SK-N-SH cell line, which may either remain undifferentiated or be developed into cells that resemble

neurons. When SH-SY5Ycells are differentiated using differentiation-provoking agents, they are released from the cell cycle and change their morphology to resemble that of primary neurons. These differentiated cells have smaller cell bodies, repeatedly polarized neuritis, excitable membranes with distinct properties (like potassium conductance), and repeatedly polarized cell bodies (Lopez-Suarez et al. 2022).

Differentiated SH-SY5Ycells are useful in vitro models to study cellular and mitochondrial morphological and bioenergetic alterations in mitochondrial dysfunction-related pathologies, including neurodegenerative diseases (Simões et al. 2022).

Undifferentiated SH-SY5Ycells may be employed to determine how neurotoxicants affect gene expression by measuring target mRNA or protein using large-scale methods or tailored research. Studies have shown that undifferentiated cells are more vulnerable to oxidative stress caused by toxins used to simulate Parkinson disease (PD) in vitro (Lopez-Suarez et al. 2022).

SH-SY5Y cells undergo both genetic and chemical modifications to mimic the pathological properties of PD. Nevertheless, many cellular processes can be investigated by using this cell line (drug screening, oxidative stress, apoptosis, mitochondrial alteration, and autophagy) (Ferrari et al. 2020).

2. Materials and Methods

The experimental work was performed in the Postgraduate lab. and the rats were housed in the Animal House/ Department of Pharmacology at the College of the Medicine /University of Babylon from (December 2022 - April 2023).

2. 1. Materials

2.1.1. Chemicals

The list of chemicals utilized in this investigation may be found in Table 2.1

Table 2.1: The chemicals utilized in this study

No.	Chemicals	Company/ Country
1.	Absolute Ethanol solution	Scharlan/ Spain
2.	Alcohol spray (ethanol 70%)	Iraq
3.	Diethyl ether solution	Laboratory reagents, India
4.	Dimethyl sulfoxide (DMSO)	Roth /Germany
5.	Fetal bovine serum (FBS.)	Gibco /U.K.
6.	Gentamicin (80 mg vial)	The Arab Pharm/ Jordan
7.	MTT dye powder	Roth /Germany
8.	Olive oil	Cesar Spain
9.	Phosphate buffer saline	Hi Media Lab/ India
10.	Rotenone Powder vial	Med Chem Express/USA.
11.	(RPMI-1640) powder	Gibco/U.K.
12.	Sinemet (Levodopa/Carbidopa) tablet	MDS./ Swiss
13.	Trypsin- Ethyl diamine tetraacetic acid (EDTA) powder	U.S. biological/U.S.A.

2.1.2. Equipment and Instruments

The equipment and instruments employed in this research with their suppliers are listed in Table 2.2.

Table 2.2: Instruments and equipment of the study:

No.	Equipment/ Instruments	Company/ Country
1.	Autoclave	Jeiotech/ Korea
2.	Automatic micropipettes (different sizes)	DRAGON MED/ Germany
3.	Cell culture flask (25ml)	SPL/Korea
4.	Cell culture plate (96- wells)	SPL/Korea
5.	Centrifuge	Hitachi, Germany
6.	Digital Rotarod	Bionic Mobin/ Iran
7.	Distiller	ROWA/ Germany
8.	Double distillation water stills	GFL/ Germany
9.	Electric oven	Memmert/ Germany
10.	Electronic scale	Sensor Disk Technology/ Chin
11.	ELISA (reader/ washer/ printer)	Haman/ Germany
12.	Eppendorf plastic tubes	Sun/ Jordan
13.	High-speed cold Centrifuge	Eppendorf/ Germany
14.	Homogenizer drill	China
15.	Incubator	Memmert/ Germany
16.	Inverted microscope	T.C Meiji techno/ Japan
17.	Laminar airflow cabinet	Labtech/ Korea
18.	Liquid nitrogen container GT38	Air Liquide /France

19.	Magnetic stirrer	Labinco /Netherland
20.	Micro-centrifuge	Memmert /Germany
21.	Micropipettes (different volumes)	Eppendorf/ Germany
22.	Microscope	Leica, Germany
23.	Millipore filter (0.45, 0.22μm)	Biofilm /Australia
24.	Plastic cassette for tissue processing	Anveon Technologies, India
25.	Refrigerator	Concord/ Lebanon
26.	Rotary evaporator	Laborota/Germany
27.	Sensitive balance	Sartorius/ Germany
28.	Soxhlet extractor	Duran Germany
29.	Spectrophotometer	Jenway/ England
30.	Surgical set	China
31.	Water bath	Memmert/ Germany
32.	Whatman filter paper	Merck/ Germany
33.	Vortex	Kottermann/Germany

2.1.3. Kits:

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

Table 2.3: ELISA kit.

NO.	Kit	Company Country
1.	Human interleukin- 1Beta(IL-1β)/ ELISA kit	Elabscience / USA
2.	Malondialdehyde (MDA) /ELISA kit	Elabscience/U.S.A.
3.	Rat interleukin- 1Beta(IL-1β)/ ELISA kit	Elabscience / USA
4.	Total antioxidant capacity (TAOC)/colourimetric assay kit	Elabscience/U.S.A.

2.2.In vitro:

2.2.1.Study Design

2.2.1.1. Cytotoxicity Assay of Rotenone in Cell Line

A pilot study was done to choose the appropriate concentration of ROT. In 96 tissue culture plates, SH-SY5Y cell lines were sown and marked. Four repetitions of each concentration of rotenone were employed for the Cell, and four repetitions served as the control group. The rotenone was applied to all cells at serial dilutions ranging from (0.6 to 40 µg/ml) the plate was covered with a self-plastic lid. Following a 24-hour incubation period, the proliferation of the cell lines was evaluated using an MTT assay to determine cytotoxicity (Han et al. 2022).

2.2.1.2. Cytotoxicity Assay of Ethanolic Extract of *B.officinalis* on SH-SY5Y cells

In 96 tissue culture plates, SH-SY5Ycell lines were sown and marked. Four replicates of each concentration of ethanolic extract were employed, and four untreated replicates served as the control group. *B.officinalis* extract were added to cells at serial dilutions ranging from (31.25 to 500 µg/ml), the plate was covered with a self-plastic lid. Following a 24-hour incubation period, the proliferation of the cell lines was evaluated using an MTT assay to determine the cytotoxicity (Moliner et al. 2022)**.**

2.2.1.3. Study the Effect of Ethanolic Extract of *B.officinalis* and Levodopa/Carbidopa (L/C) on SH-SY5Y Cells

SH-SY5Y cell lines were seeded in 96 tissue culture plates. All cells were pretreated with different concentrations of *B.officinalis* extract (BOE) at serial dilutions ranging from 31.25 to 500 µg/ml (four replicates were used for BOE) (Moliner *et al*., 2022) and four replicated of (L/C) (10 µg/ml) along with four replicates as a control group for 2 hours at 37°C then challenged with ROT (20 µg/ml), the plate was covered with a self-plastic lid. After a 48-hour incubation period following the exposure period, the cell lines were taken for immunoassay by ELISA method using MDA, IL-1β and TAOC Colorimetric assay .

2.2.2. Preparation of Reagents and Solutions

2.2.2.1. Rotenone Preparation for SH-SY5Y Cells

Preparation of stock solution of rotenone by dissolving 10 mg of powder in 0.5 ml of DMSO and 1.5ml of serum-free RPMI to produce a final concentration of 20µg/ml. The mixture is then filtered through a 0.22 µm Millipore filter to remove any contaminants (Han et al. 2022).

2.2.2.2. *B.officinalis* Preparation for SH- SY5Y Cells

Flowers of *B.officinalis* were collected from local markets (Babylon, Iraq) . About 30g of air-dried flowers grained and dissolved in 300 ml of ethanol (95%) ,the extract was done using soxhlet for 4 hours and temperature ranged between 80°C and 85°C.Then a rotary flash evaporator was used to remove the solvent from the extract, and the resultant extract was stored at 20° C until use. Preparation of stock solution of ethanolic extract of *B.officinalis* was done by dissolving 5 mg of the dried extract in 5 ml of serum-free RPMI to produce a final concentration of 1000µg/ml. The mixture was then filtered through a 0.22 µm Millipore filter to remove any contaminant (Moliner et al. 2022)(Yaghmour et al. 2021).

2.2.2.3. L-dopa/Carbidopa (L/C) Preparation for SH- SY5Y Cells

Preparation of (L/C) stock solution was done by dissolving one tablet of (L/C) 250/25 mg in 5 ml of serum-free RPMI to produce a final concentration of 10µg/ml. The mixture was then filtered through a 0.22 µm Millipore filter to remove any contaminants (Kesh et al. 2021) (Allen et al. 2013).

2.2.2.4. Phosphate Buffer Saline (PBS)

According to the BioWorld manufacturer manual, the PBS was prepared by dissolving only one packet in 500 ml of deionized distilled water (DDW) with continuous stirring by a magnetic stirrer at room temperature resulting in a PH. value of 7.45. Autoclaving is required for complete sterilization and stored in a closed bottle until used to keep it sterile.

2.2.2.5. Trypsin- Ethylenediaminetetraacetic Acid (EDTA) Solution

As indicated by the manufacture (U.S. Biological), a weight of 10.1 gm of trypsin EDTA powder dissolved in 0.9 Liter of double distilled water (DDW) with continuous mixing at room temperature. PH value of 7.2 should be reached and complete the volume to 1 Liter by DDW, the solution was sterilized using Millipore filters 0.22 μm .After that, the solution was kept at (- 20C˚) temperature (Phelan and May, 2017).

2.2.2.6. Preparation of MTT Solution

The (3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) solution was prepared by dissolving 5 mg of MTT powder in 1 ml of DDW to get final concentration of (5 mg/ml). The solution is then sterilized by filtration through a 0.2 μm Millipore filter and placed in a dark, secure container. The MTT solution should be protected from light and stored at 4 °C or -20°C until use (Kamiloglu et al. 2020) (Figure 2.1) .

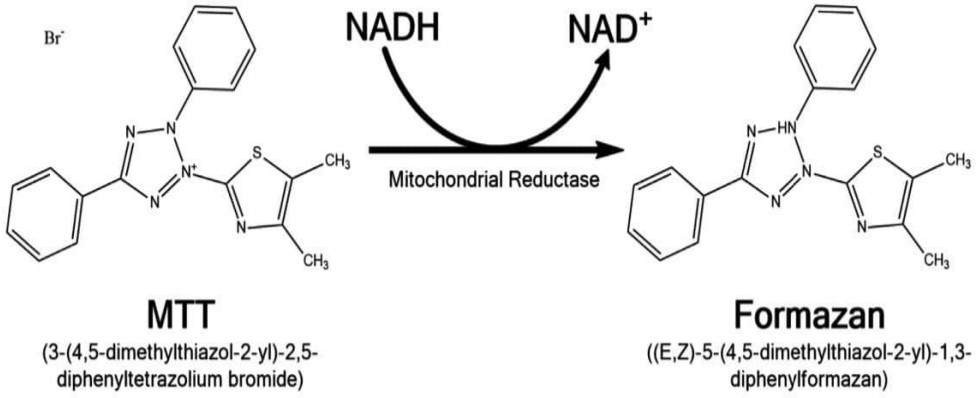

Figure 2.1. Principle of MTT Assay

Procedure of cytotoxicity assay using MTT

1- After the end of the drug exposure period, the media was taken out of the wells, and the cells were then rinsed with PBS. A blank control was performed to evaluate nonspecific formazan conversion.

2- To get a final concentration of 0.5 mg/ml, 10.8 ml of medium was mixed with 1.2 ml of MTT solution (5 mg/ml). The resultant solution was then poured (200µl) into each well.

3- After three hours of incubation at 37°C ,100µl of DMSO was added to each well to dissolve the resulting formazan crystals after removing the supernatant.

4- The plate was incubated for 30 minutes at room temperature until the purple crystals disintegrated and the cells lysed.

5- Using a microplate reader, absorbance was measured at 570 nm (Langdon 2011) .

2.2.2.7. Preparation of Serum-Free Medium

Liquid Roswell Park Memorial Institute (RPMI-1640) medium: Liquid RPMI-1640 medium was prepared according to the Gibco product manual from RPMI-1640 medium powder as the following: 10.43 gm of RPMI-1640 medium powder was dissolved in 0.9 L of DDW in a volumetric flask. Additional constituents include: 2 gm sodium bicarbonate powder as needed, and 80 mg of gentamicin were added with continuous stirring. The solution was completed to 1 liter by DDW, adjusting the PH. at 7.4. Use 0.4 and 0.2 μm Millipore filters to sterilize the solution under the airflow cabinet. For examination of any contamination, the prepared medium was incubated at 37 ºC overnight with continuous following up, if there was no contamination, the medium could be used or stored at 4ºC temperature until use (Phelan and May, 2017).

2.2.2.8.Preparation of Serum-Medium

Medium with serum was prepared as described in the Preparation of serum-free medium by adding 10% of fetal bovine serum (FBS).

2.2.2.9.Preparation of Freezing Medium

The freezing medium consist of 60% RPMI medium, 30% FBS and 10% DMSO (Phelan and May, 2017).

2.2.3. Preparation of Cell Lines

2.2.3.1. Thawing of SH-SY5YCell Lines

The SH-SY5Ycell line was purchased from National Cell Bank, Tehran, Iran. With care, the frozen SH-SY5Ycell line vial was removed from the liquid nitrogen container and put right into a beaker with sterile DDW. pre-warmed to 37°C. Before the ice floccules entirely disintegrated, the vial was removed from the water and cleaned with 70% ethanol. The vial's cell suspension was immediately

transferred into a 15ml sterilized plastic centrifuge tube with 10 ml of the pre-warmed serum-free medium while pipetting under a laminar flow cabinet. The supernatant was aspirated and decanted after 5 minutes of centrifugation at 1000 rpm. The cells pellet was re-suspended in 5 ml of warm (37 °C) serum medium before being transferred into a cell culture flask of 25 ml. The cells were then incubated at 37 °C, and the serum medium was changed the next day (Phelan and May, 2017).

2.2.3.2. Sub-culturing of SH-SY5YCell Lines

1- Using an inverted microscope, the SH-SY5Ycells are checked and evaluated to make sure they are healthy and free of contaminants.

2- Cleaning the surface of the work area with 70% ethanol sanitizes the laminar flow.

3- The growing medium were removed from the flask, use a pipette, and the monolayer is then washed with enough PBS to verify that all of the growth media has been taken out of the flask.

4- An adequate volume of trypsin/EDTA solution was added to the flask, and it is then incubated at 37°C for the required amount of time (usually 2–10 min) for the cells to separate from the flask's internal surface.

5- An inverted microscope was used to check the cells to ensure they were all detached and suspended.

6- Addition an equal volume of serum-containing medium was added to the flask, the trypsin is inactivated.

7- The cell suspension is then split into two flasks, and each flask is labelled with the name of the cell line and the date.

8-The cell line was incubated at 37°C for 24 hr (Meleady and O'Connor, 2005).

2.2.3.3. Harvesting of SH-SY5Ycell line

Trypsin enzymes are used in the harvesting process to separate adherent cells from the surface of a cell culture flask. The growing medium inside the vessel was aspirated first and thrown away. The cells were washed twice with PBS. Then the vessel was filled with the enzymatic harvesting solution. After 15 minutes, adding the culture medium containing serum stopped the proteolytic reaction. Trypsin and ethylene-diaminetetraacetic acid (EDTA) solutions with varying trypsin and EDTA concentrations were used to harvest the cells from the tissue culture flasks (Viazzi et al. 2015).

2.3.In vivo

2.3.1. Animals

In this study, 70 male adult Albino rats whose weights range between 200-300 grams were used. The rats were housed in the Animal House of the College of Medicine/ University of Babylon. They were kept in 14 cages, 5 rats in each cage at 25° C temperatures with 14 hours in daylight and 10 hours in darkness cycle with water and food ad libitum. After two weeks of adaptation, the animals were randomly divided into seven groups, 10 rats per each group.

2.3.2. Study Design

2.3.2.1. The Pilot Study of Rotenone for Rats

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

- Group 1: the negative control

- Group 2: Each rat received 1.5mg/kg of rotenone by subcutaneous route every other day for 21 days
- Group 3: Each rat received 2.5mg/kg of rotenone by intraperitoneal (I.P) route every other day for 21 days
- Group 4: Each rat received 2mg/kg of rotenone by the I.P. route every day for 21 days. The results showed that group 2 had the highest mortality rate, and group 3 had the clearest Parkinsonism symptoms.

2.3.2.2. Study Design in Rats

The 70 rats were randomly divided into seven groups, with ten animals in each group:

- Group 1: healthy control group.
- The other sixty rats were induced with Parkinsonism by rotenone IP 2.5mg/kg every 48hr for 21 days and divided as follows:

1. Group 2: untreated Parkinson disease rats.
2. Group 3: treated with 10mg/kg of L-dopa/Carbidopa tablet orally by gastric gavage every day for 21 days
3. Group 4: treated with 62.5mg/kg of ethanolic extract of *B.officinalis* (BOE) orally by gastric gavage every day for 21 days
4. Group 5: treated with 125mg/kg of (BOE) orally by gastric gavage every day for 21 days
5. Group 6: treated with 250mg/Kg of (BOE) orally by gastric gavage every day for 21 days
6. Group 7: treated with 500mg/kg of (BOE) orally by gastric gavage every day for 21 days

- The rats were weighed on days 0, 10, and 21 of the experiment.

- At day 22, 24 hours following the last dose, behavioral tests were carried out to compare the progression of Parkinsonism and the efficacy of the treatment. Each rat was put on a rotarod for three trials and then left in the open field for ten minutes and three minutes for force gripping test . A video camera was used to record every behavior.
- The mid-brain samples from each animal were then taken to analyze the tissue levels of interleukin-1β (IL-1β), malondialdehyde (MDA) and total antioxidant capacity (TAOC).

2.3.2.3. Decapitation of Rats Brain and Tissue Samples Preparation

Each rat was sacrificed on the 22nd day, the skull was dissected posteriorly from the foramen magnum, and the brains were removed. The brain, cerebellum, and olfactory pulps were carefully removed from the skull, along with the midbrain and forebrain being removed, dissected, rinsed with phosphate buffer solution, and weighting.

Figure 2.2 below illustrates midbrain brain, olfactory bulbs, right and left hemispheres and cerebellum of rat.

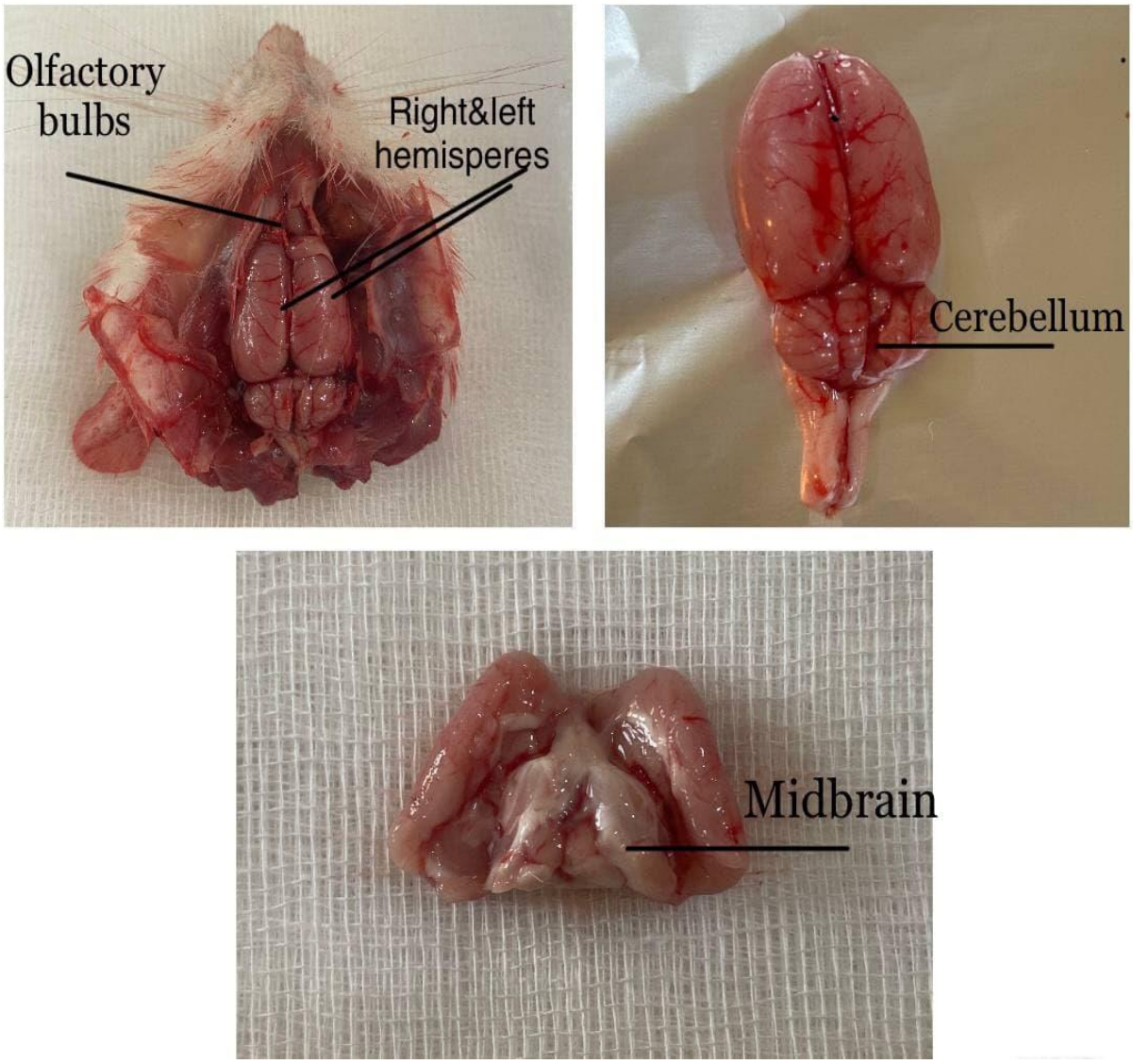

Figure 2.2. Brain dissection

2.3.2.4. Brain's Samples Preparation

- Brain Homogenization: Remaining blood was washed away using a pH 7.4 pre-chilled PBS.
- After being weighed, the brain was homogenized using a homogenizer on ice in PBS (pH = 7.4).
- Freeze at -20◦C for 20 minutes.

2.3.3. Preparation of Drugs and Chemicals

2.3.3.1. Rotenone Preparation for Rats Model

To induce Parkinsonism, rats were intraperitoneally (IP.) injected with (2.5 mg/kg Body Weight). Stock solution of 125 mg/ml of ROT was prepared by dissolving 125 mg of rotenone in 1 mL of dimethyl sulfoxide (DMSO) of a 50X stock solution of dimethyl sulfoxide (DMSO) (Javed et al. 2016). A fresh solution was made twice a week, by diluting 40 µl of the stock solution in 1960 µl of olive oil, mix the solution well before each use. Each rat received 1ml/kg of the produced solution (Mbiydzenyuy et al. 2018).

2.3.3.2. L-dopa/Carbidopa (L/C) Preparation for Rats Model

One tablet of (L/C) 250/25 mg was grinded and dissolved in (25ml) of D.W , to get final concentration of (10 mg/kg), fresh daily dose was prepared for each rat in (L/C) group with shaking before oral administration (Priyanga *et al.*, 2017).

2.3.3.3. *Borago.officinalis* Extract Preparation for Rats Model

Flowers of *B.officinalis* were collected from local markets (Babylon, Iraq) . About 30g of air-dried flower grained and dissolved in 300 ml of ethanol (95%) ,the extract was done using soxhlet for 4 hours and temperature ranged between 80°C and 85°C.Then a rotary flash evaporator was used to remove the solvent from the extract, and the resultant extract was stored at 20° C until use. Water was used to dilute the extracts just before the experiment. Then, 5 grams of (BOE) were dissolved in 10 ml of distilled water, yielding a final concentration of 500 mg per ml (Moliner et al. 2022)(Yaghmour et al. 2021) (picture 2.3).

Figure 2.3: *B.officinalis* dried flower

2.3.4. Behavioral Equipment

2.3.4.1. Rotarod Apparatus

The motor capacity and coordination of rodents are assessed using this behavioral test. Rats must balance on a rotating cylinder with a variable speed to complete the task. The rotating cylinder was used to inspect each rat for three minutes at a speed of 20 rpm. Rat performance was evaluated for motor coordination by counting the number of revolutions. After each test, the device was sterilized with 70% ethanol (Rao et al. 2019) (Figure 2.4).

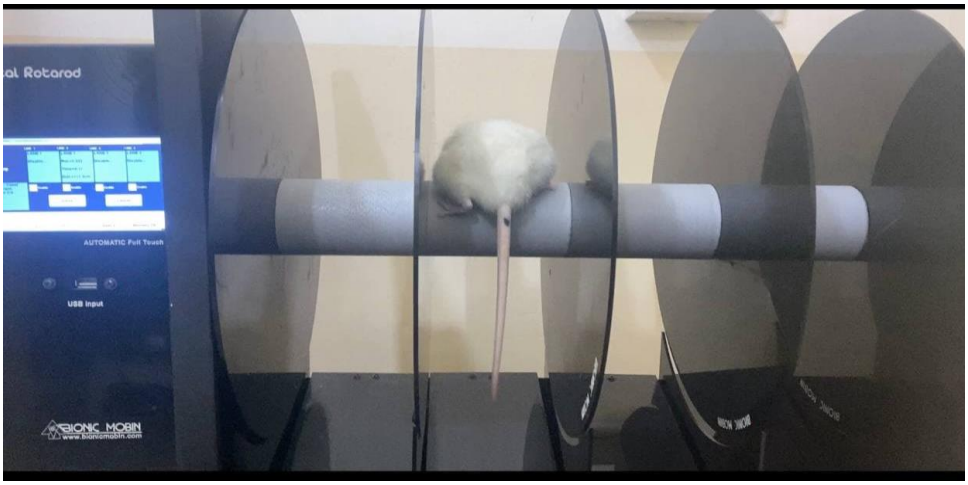

Figure 2.4. Rotarod Apparatus

2.3.4.2. Open Field Box

This wooden box (100 x 100cm) was made by researchers, according to (Martinez-Gonzalez et al. 2004), consisting of a square floor divided by thin white lines into 100 equal squares (Figure 2.5). 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 squares crossing throughout the test time was referred to as crossings, and it is used to determine the animals' locomotors 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 number of visits to the open field center is used to assess risk-taking behavior. The term grooming refers to the overall amount of period spent grooming (Figure 2.6). The device was sterilized with 70% ethanol after each test. A video camera recorded all behaviors (Shehata et al. 2020) .

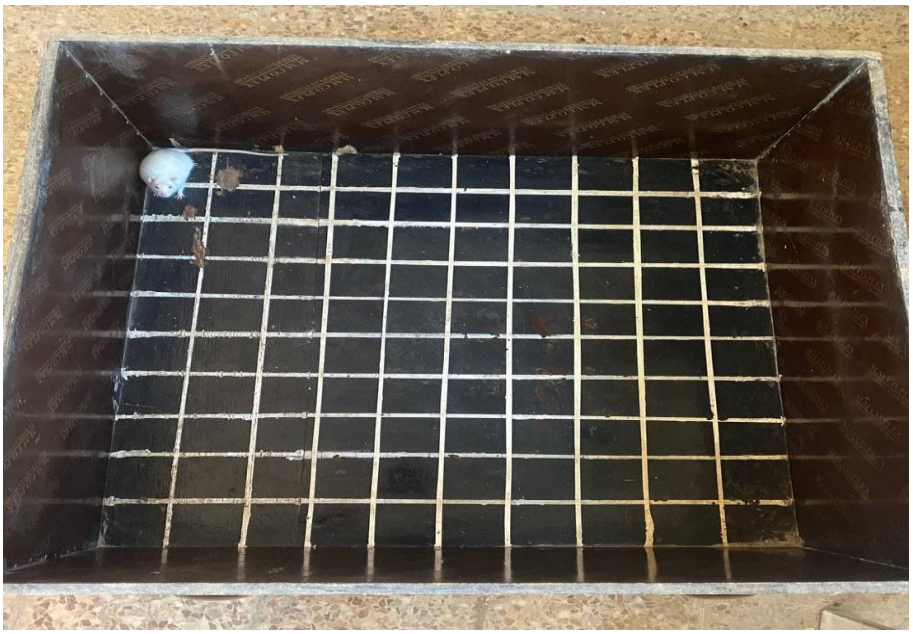

Figure 2.5. Open field box

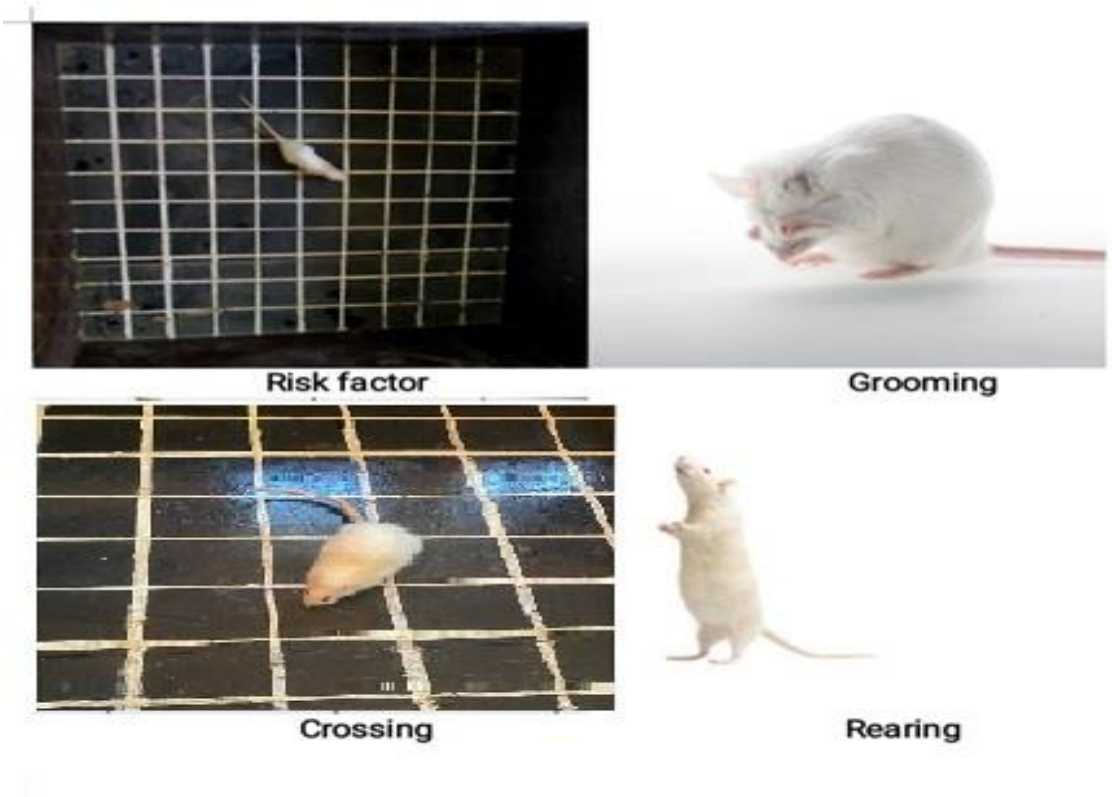

Figure 2.6. Open Field Box, Representation of: A. Risk factor, B. Grooming, C. Crossing and D. Rearing

2.3.4.3. Force Gripping Apparatus

A wooden chamber containing a stainless beam (1m x 60cm x60cm) was built by researcher. The rat was placed on a beam, and the latency time to fall was recorded by a video camera (Abdelkader et al. 2020) (Figure 2.7).

Figure 2.7. Force Gripping Apparatus

2.4. Biochemical Assessments

2.4.1. Malondialdehyde (MDA) ELISA Kit

The plate had been pre-coated with MDA antibody. The sample's MDA was added to the wells, where it interacted with Abs. The MDA Antibody that had been biotinylated was then introduced to the sample, bound to MDA The "Streptavidin-horseradish peroxidase (HRP)" then bound the biotinylated MDA antibody. After incubation, "Streptavidin-HRP" that was not bound was removed through washing. The substrate solution was added, and the colour changed according to how much rats or human MDA was present. The process was stopped by adding an acidic stop solution, and the absorbance at 450 nm was determined. Standard Curve Range: 0,05-10nmol/ml; Sensitivity: 0.023nmol/ml (Elabscience, 2020).

2.4.2. Interleukin -1β(IL–1β) ELISA Kit

The plate has already been pre-coated with IL-1β antibody. The sample's IL-1β is delivered to the wells, where it interacts with the antibodies. The IL-1β antibody biotinylated in the sample is subsequently introduced and binds to IL-1β. The Streptavidin-HRP then linked the biotinylated IL-1β antibody. During the washing phase following incubation, all unbound Streptavidin-HRP is removed. The substrate solution is then added, and the colour will change depending on how much Rat IL-1β is present. By adding a stop solution and at 450 nm measuring the absorbance, the process is stopped (Elabscience, 2022) .

2.4.3.Total Antioxidant Capacity (TAOC) Colorimetric Assay (ABTS, Enzyme Method)

The principle of the 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method for determining the T-AOC is as follows.

ABTS is oxidized to green ABTS by appropriate oxidant, which can be inhibited if there exist antioxidants. The T-AOC of the sample could be determined and calculated by measuring the absorbance of ABTS* at 414 m or 734 nm. Trolox is an analog of vitamine E (VE) and had a similar antioxidant capacity to that of VE. Trolox is used as a reference for other antioxidants. For example, the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with the same concentration is showed by the ratio of its antioxidant capacity to Trolox antioxidant capacity.

2.5. Statistical Analysis

The SPSS version 26 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 $p\text{-}value \leq 0.05$ was considered significant.

3.1. In Vitro

3.1.1. MTT analysis:

3.1.1.1. Cytotoxicity Assay of Different Concentrations of Rotenone in Cell Line

There was a significant decrease (P-value<0.05) in the viability of the SH-SY5Ycells in all rotenone concentration except at (5 and 10µg/ml) when compared with the control group after an incubated period of 24 hours at 37°C (Figure 3.1).

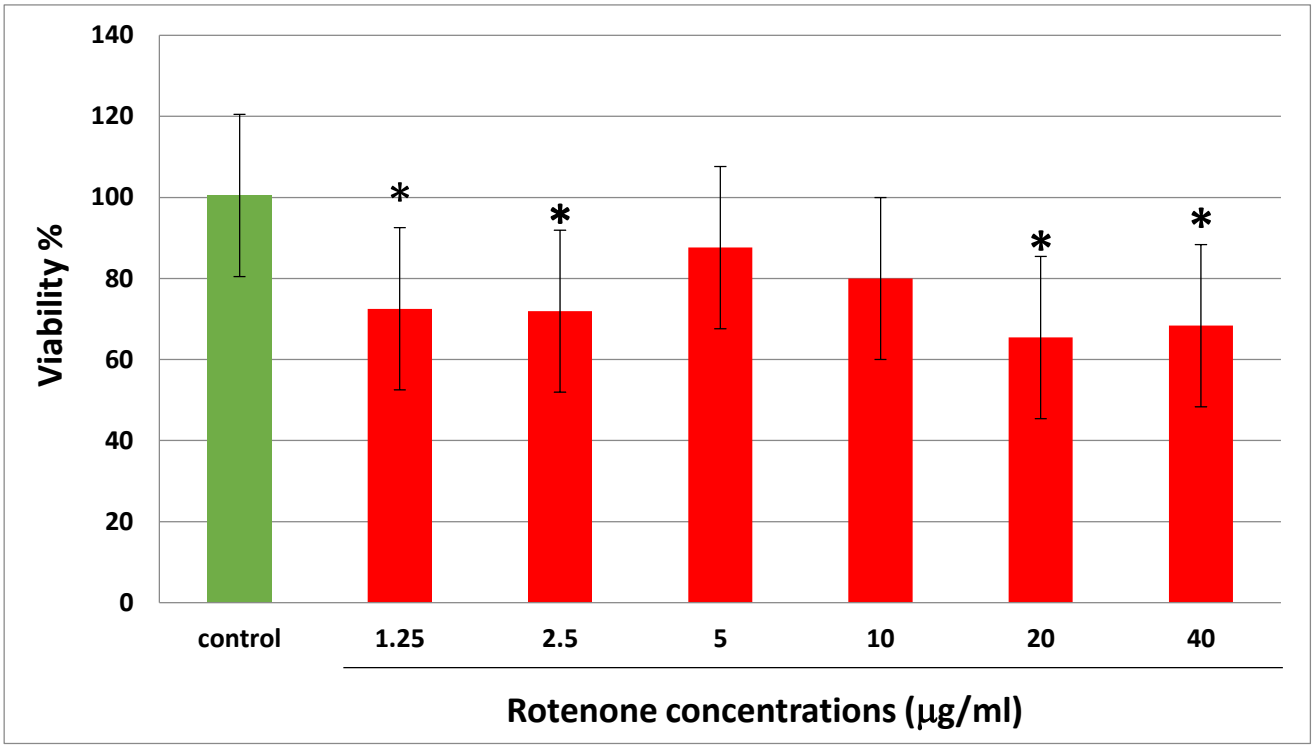

Figure 3.1: Effects of different concentrations of rotenone on the viability of SH -SY5Y cell line measured using MTT assay.

***=significant decrease (p-value <0.05) as compared to control group.**

3.1.1.2.Cytotoxicity Assay of Different Concentrations of Ethanolic Extract of *Borago.officinalis* in Cell Line

There was a significant decrease (P-value<0.05) in the viability of the SH-SY5Ycells in (BOE) concentrations at (500 and 250µg/ml) as compared with the control group after an incubated period of 24 hours at 37°C (Figure 3.3).

The undifferentiated SH-SY5Y cells below in (Figure 3.2)

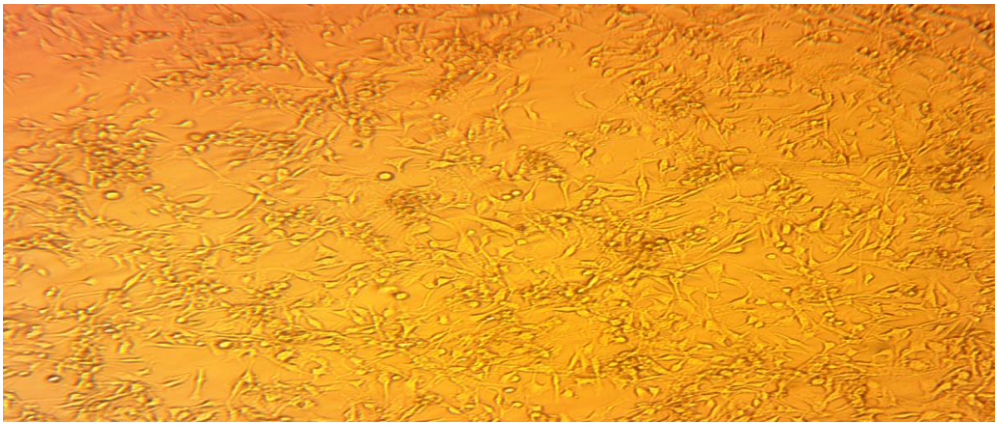

Figure 3.2: SH-SY5Y cells

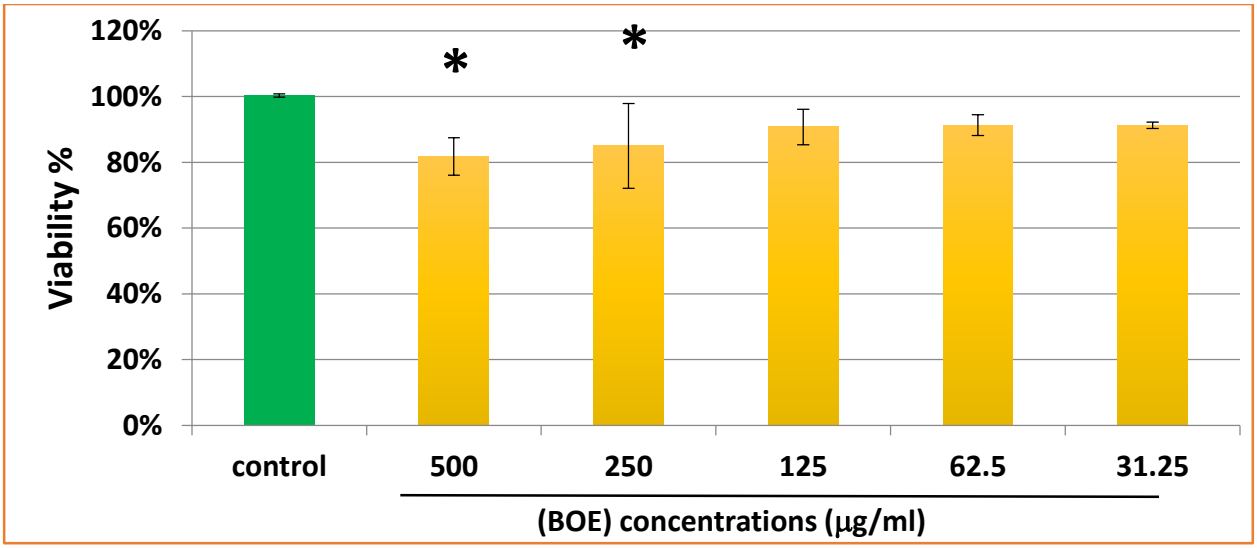

Figure 3.3: Effects of different concentrations of *B.officinalis* extract (BOE) on the viability of SH -SY5Y cells measured using MTT assay.

***=significant decrease (p-value <0.05) as compared to control group.**

3.1.1.3.Study the Effects of Ethanolic Extract of *Borago.officinalis and* L-dopa/Carbidopa on SH-SY5Y Cells

Cells viability level significantly decreased (p-value<0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) treated group and in cells treated with all (BOE) concentrations as compared with control group. Whereas, cells viability were significantly increased (p-value <0.05) in (L/C) treated group and in cells treated with (BOE) as compared with ROT group (Figure 3.4) .

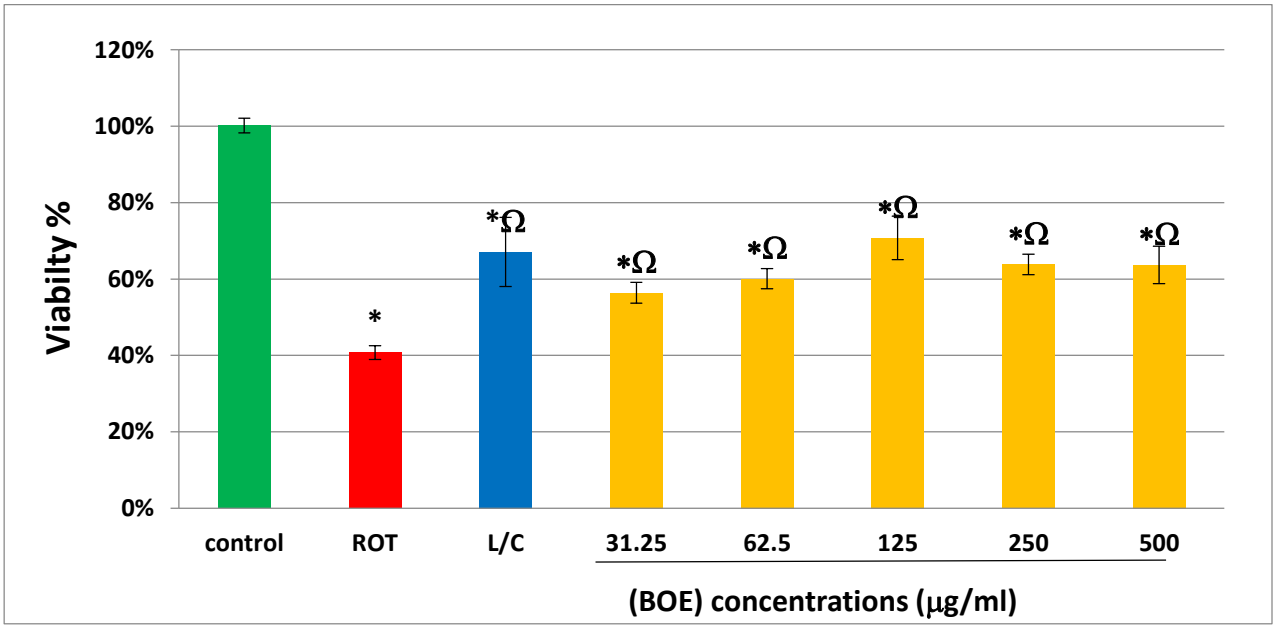

Figure 3.4: Effects of different concentrations of *B.officinalis* extract (BOE) on the viability of SH-SY5Y cells treated with ROT. (L/C): (10µg/ml) , ROT: (20µg/ml).

***=significant decrease (p-value <0.05) as compared to control group.**
Ω=significant increase (p-value <0.05) as compared to ROT group.

3.1.2. Biochemical Study

3.1.2.1.Malondialdehyde (MDA) levels

Malondialdehyde level significantly increased (p-value <0.05) in ROT group and in cells treated with (BOE) concentrations of (31.25,62.5,250 and 500 µg/ml) and there was no significant difference in L-dopa/Carbidopa (L/C) group and in cells treated with (BOE) concentration of 125 µg/ml as compared with control (healthy) group. Whereas, MDA significantly decreased (p-value <0.05) in cells treated with (L/C) and (BOE) concentrations of (31.25, 125,250 and 500µg/ml) as compared to ROT group. Additionally, there was a significant increased in cells treated with (BOE) concentration (31.25, 62.5 and 250µg/ml) ,but there was no significant difference in cells treated with (BOE) concentrations (125 and 500µg/ml) as compared to (L/C) group (Figure 3.5).

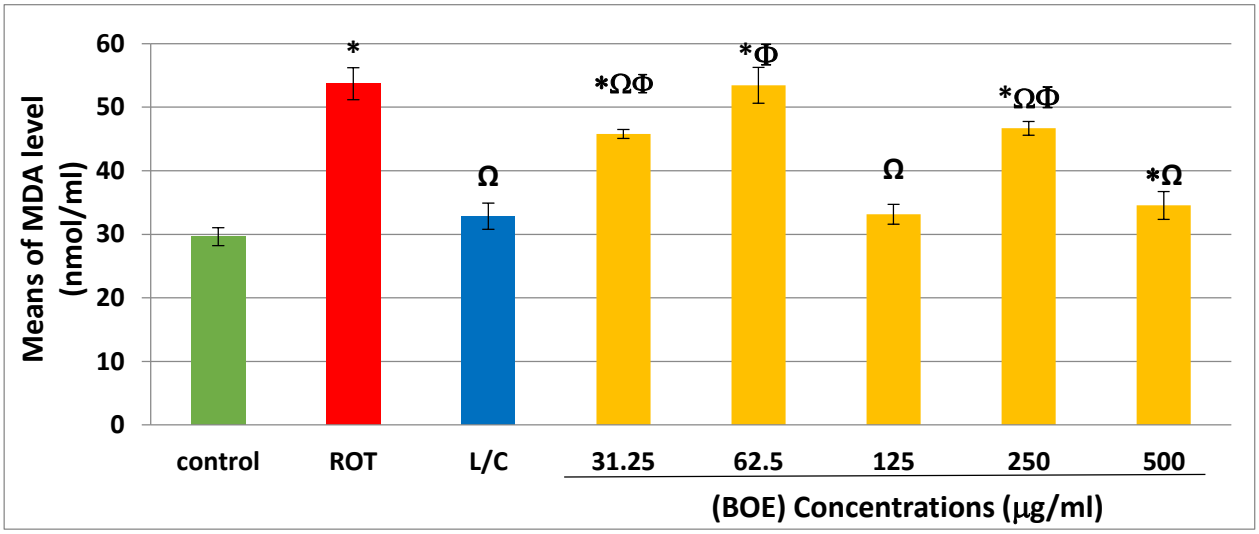

Figure 3.5: Means of MDA levels ± SEM of all groups. control: healthy untreated group, ROT: (20 µg/ml), (L/C): (10µg/ml).

***=significant increase (p-value <0.05) as compared to the control group.**
Ω=significant decrease (p-value <0.05) as compared to the ROT group.
Φ=significant increase(p-value <0.05) as compared to the (L/C) group.

3.1.2.2. Interleukin-1β (IL-1β) Levels

Interleukin-1β level significantly increased (p-value <0.05) in rotenone (ROT) group and in cells treated with (BOE) concentrations of (31.25,62.5 ,250 and 500 µg/ml) and there was no significant difference in cells treated with L-dopa/Carbidopa (L/C) and (BOE) concentration (125µg/ml) as compared with control group .Whereas, IL-1β significantly decreased (p-value <0.05) in (L/C) treated group and in cells treated with (BOE) concentration of (125µg/ml) as compared with ROT group. Furthermore,IL-1β Significantly increased in cells treated with (BOE) concentrations of (31.25,62.5,250 and 500µg/ml) ,but there was no significant difference in cells treated with (BOE) concentration of (125µg/ml) as compared to (L/C) treated group (Figure 3.6).

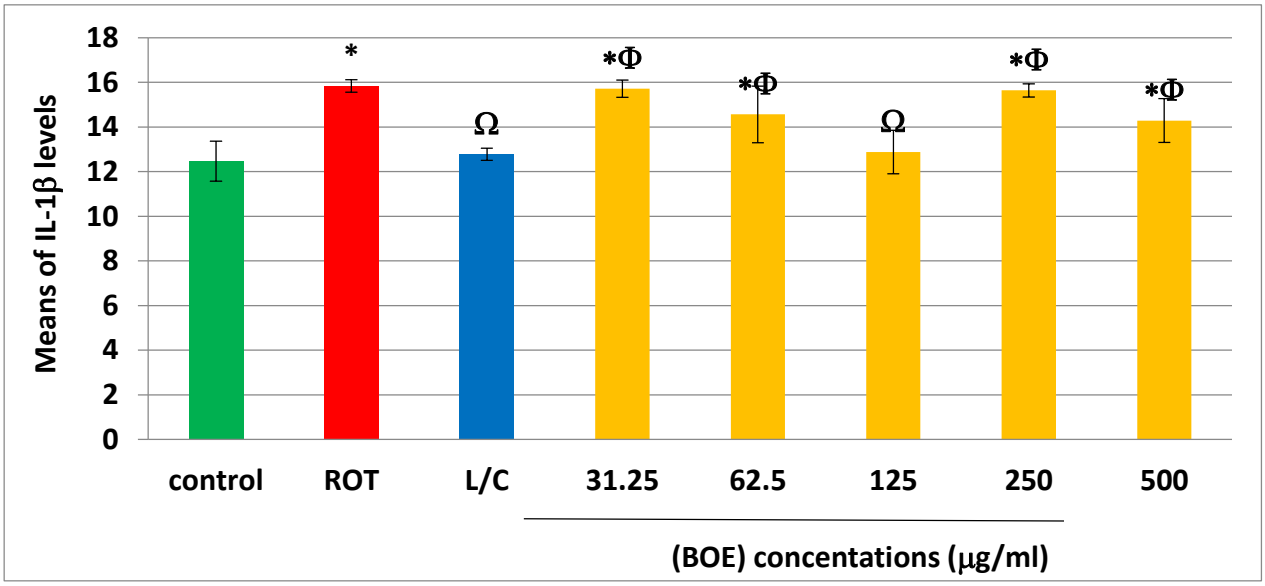

Figure 3.6 :Means of IL-1β levels ± SEM of all groups. control: healthy untreated group, ROT: (20 µg/ml), (L/C):(10µg/ml).

***=significant increase (*p-value< 0.05)* as compared to the control group.**
Ω=significant decrease (*p-value< 0.05)* as compared to the ROT group.
Φ=significant increase(*p-value< 0.05)* as compared to the (L/C) group.

3.1.2.3.Total Antioxidant Capacity (TAOC) Levels

Total antioxidant capacity level significantly decreased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) treated group and in cells treated with all (BOE) concentrations as compared to control(healthy) group. while, TAOC significantly increased (p-value <0.05) in (L/C) treated group and in cells treated with all (BOE) concentrations as compared with ROT group. Furthermore, TAOC significantly decreased (p-value <0.05) in cells treated with (BOE) concentrations of (31.25,62.5 and 250 µg/ml) and significantly increased (p-value <0.05) in cells treated with (BOE) concentration of (125µg/ml) , with no significant difference at (BOE) concentration(500µg/ml) as compared with (L/C) group (Figure 3.7).

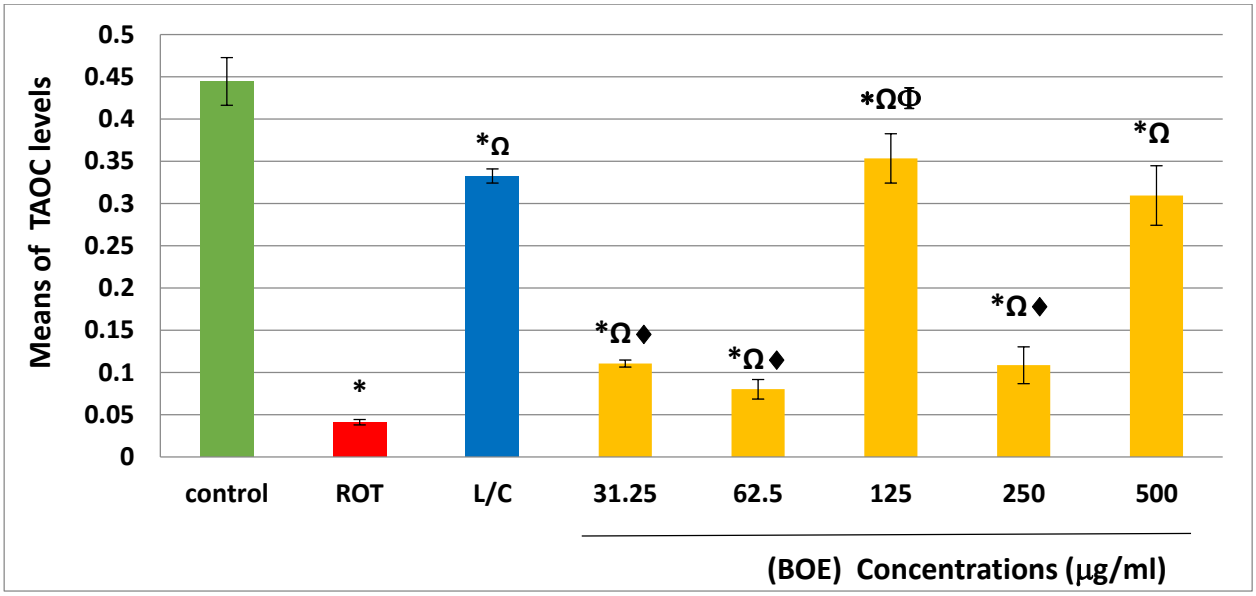

Figure 3.7:Means of TAOC levels ± SEM of all groups. control: healthy untreated group, ROT: (20 µg/ml), (L/C): (10µg/ml).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.
♦=significant decrease (p-value <0.05) as compared to the (L/C) group.

3.2.In Vivo

3.2.1. Weights of Rats

In comparison to day 0, group1 (control group, untreated and unexposed to ROT), there were insignificant differences in means of weight on day 10 and day 21. Additionally, in group 2 (untreated but exposed to ROT), the means of weight significantly decreased (p-value <0.05) on day 21 in comparison to day 0.

In group 3 (pretreated with L-dopa/Carbidopa and being exposed to ROT), in group 4 (pretreated with 62.5mg/kg (BOE) and being exposed to ROT), group5 (pretreated with 125mg/kg (BOE) and being exposed to ROT), group6 (pretreated with 250mg/kg (BOE) and being exposed to ROT) and group7 (pretreated with 500mg/kg (BOE) and being exposed to ROT) there were insignificant differences in the means of weight on day 10,21 in comparison to day o (Figure 3.8).

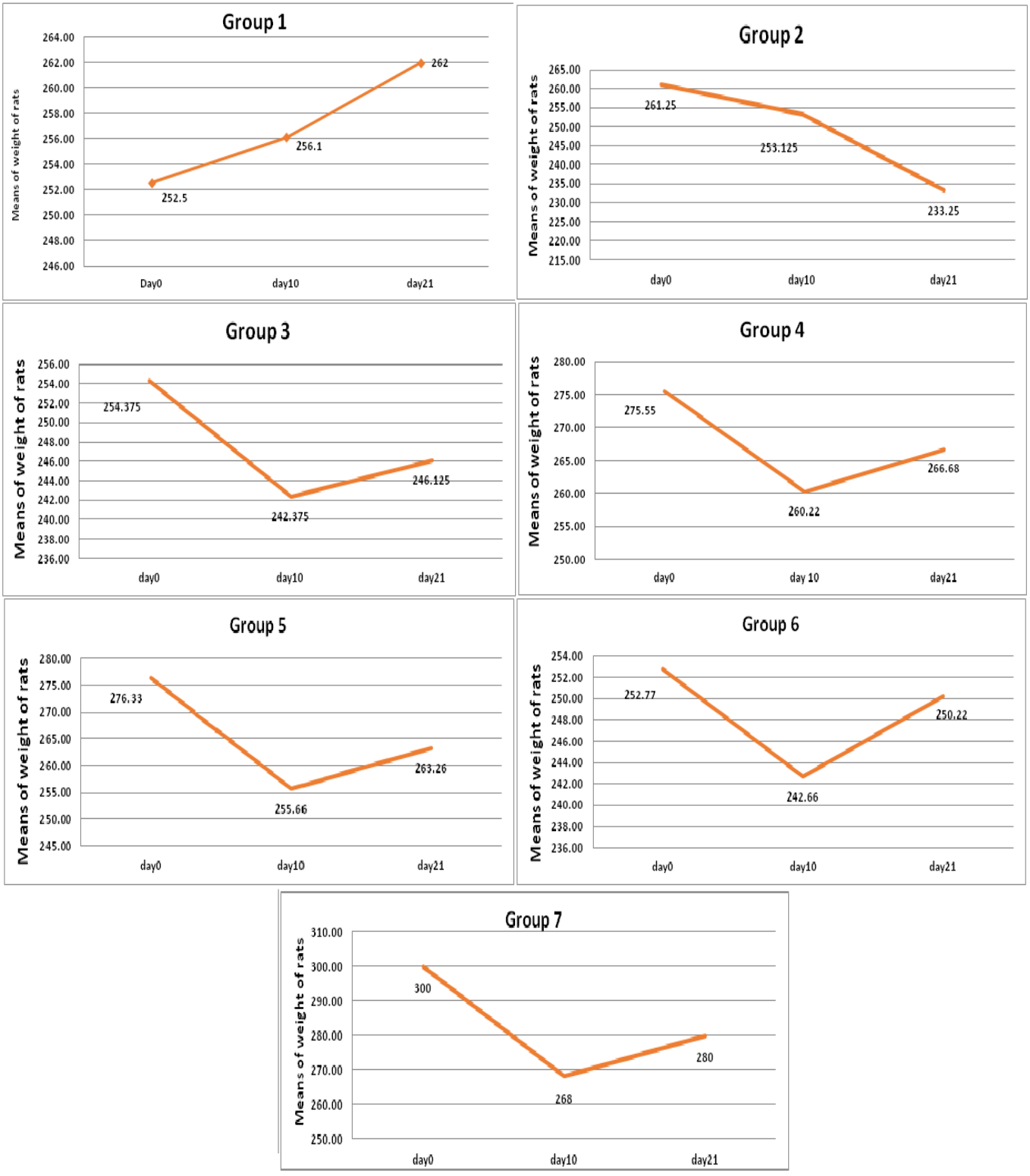

Figure 3.8. Body weight means for all groups on days 0, 10, and 21.

3.2.2. Effect of *Borago.officinalis* Extracts on Rotarod Test

3.2.2.1. Number of Rotations

The number of rotations significantly decreased (p-value <0.05) in rats treated with the rotenone (ROT), L-dopa/Carbidopa (L/C) and (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared with control (healthy/ untreated) group. The number of rotations significantly increased (p-value <0.05) in rats treated with (L/C) and (BOE) concentrations (125 ,250 and 500 mg/kg) as compared with ROT treated group. Furthermore, there was a significant increase in rotations number in rats treated with (BOE) concentrations of (125 mg/kg) as compared to (L/C) treated group (Figure 3.9).

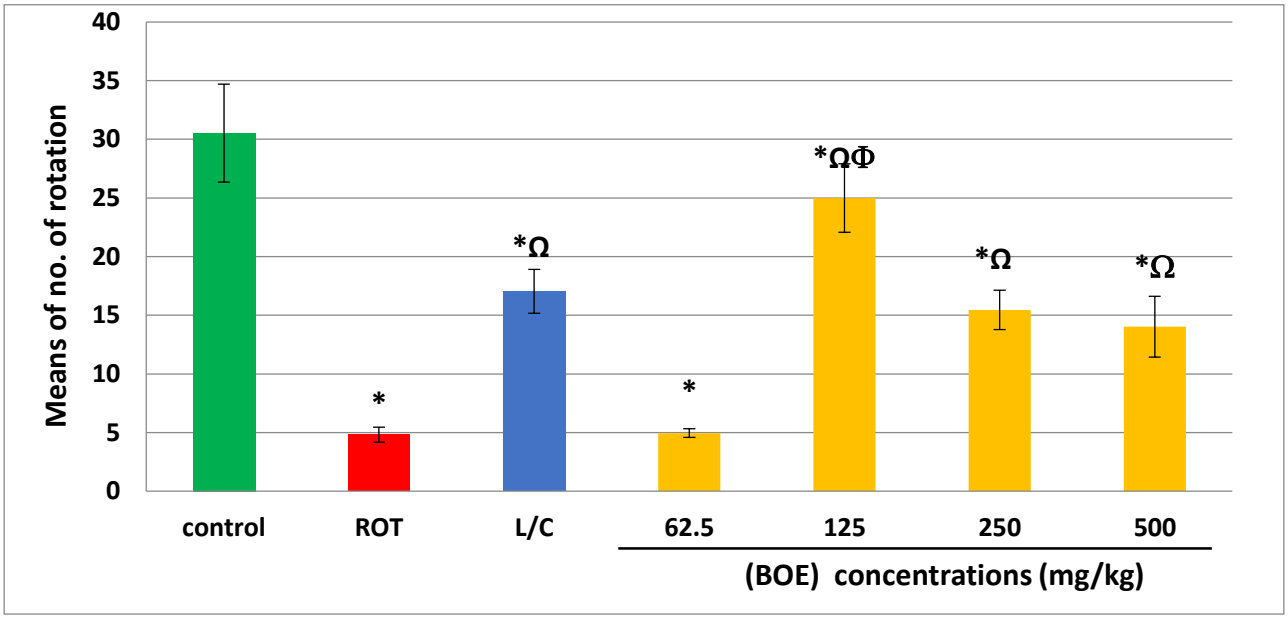

Figure 3.9:Means of number of rotation ± SEM of all groups, Control: healthy untreated group, ROT: (2.5mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.

3.2.2.2.Rotation Distance

The rotations distance significantly decreased (p-value <0.05) in the rotenone (ROT) treated group, L-dopa/Carbidopa (L/C) treated group, and in rats treated with (BOE) concentrations of (62.5 ,125 ,250 and 500 mg/kg) as compared with control (healthy/untreated) group. In contrast, there was a significant increased (p-value <0.05) in rats treated with (L/C) and (BOE) concentrations of (125,250 and 500mg/kg) as compared with ROT group .furthermore, rotation distance significantly increased (p-value <0.05) in rats treated with (BOE) concentrations of (125 mg/kg) as compared to (L/C) group (Figure 3.10).

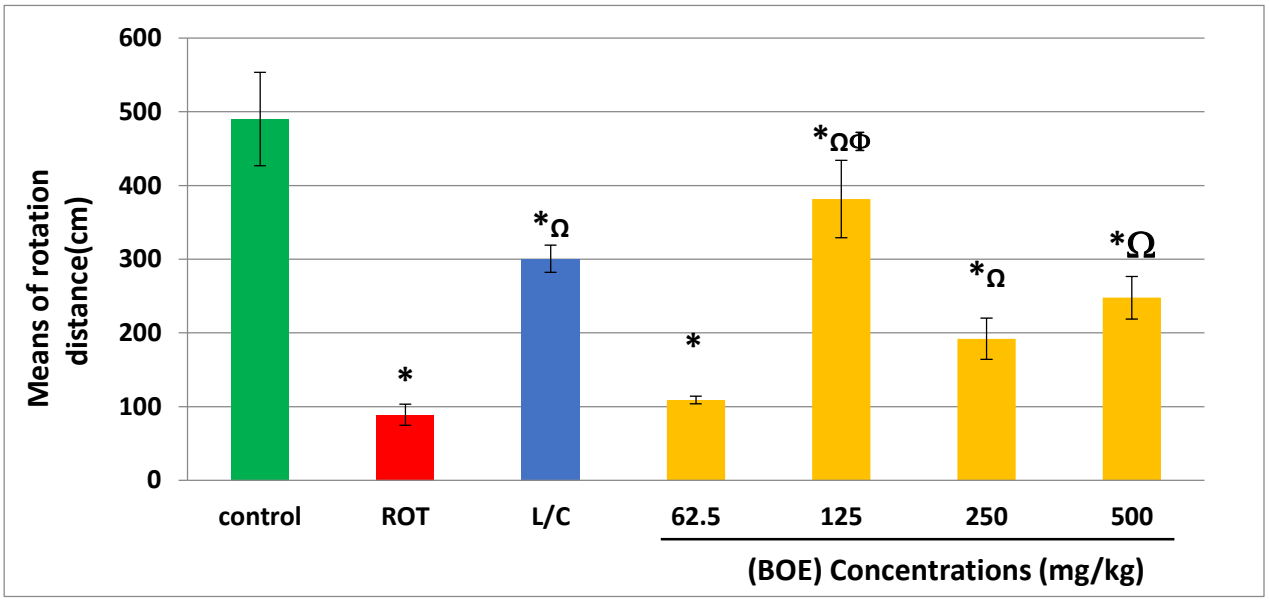

Figure 3.10: Means of rotation distance (cm) ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.

3.2.2.3.Time of Rotation

The time of rotations significantly decreased (p-value <0.05) in the rotenone (ROT) treated group, L-dopa/Carbidopa (L/C) treated group, and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to control (healthy) group. Whereas, there was a significant increased (p-value <0.05) in rats treated with (L/C) and (BOE) concentrations of (125, 250 and 500 mg/kg) as compared to ROT group. Furthermore, there was a significant decreased (p-value <0.05) in rats treated with (BOE) concentrations of (62.5,250 and 500 mg/kg), but there was no significant difference in rats treated with (BOE) concentration of 125 mg/kg in comparison with (L/C) group (Figure 3.11).

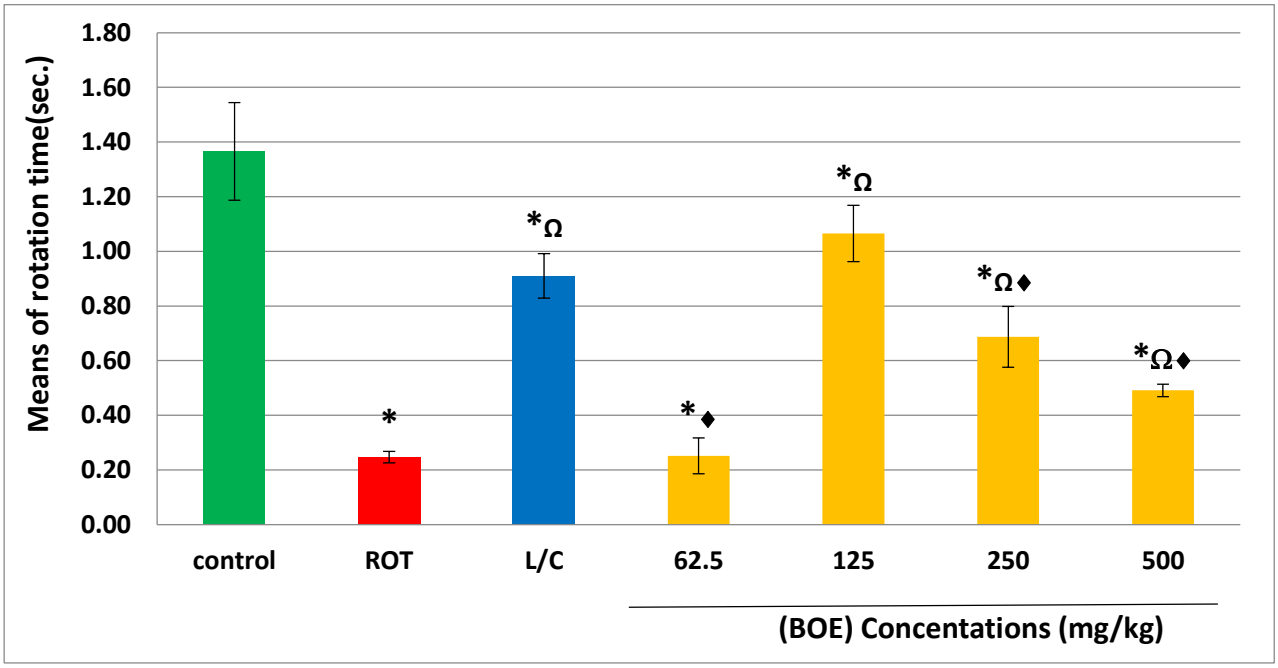

Figure 3.11: Means of rotation time (sec.) ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
♦=significant decrease (p-value <0.05) as compared to the (L/C) group.

3.2.3. Effect of *Borago.officinalis* Extracts on Open Field Test

3.2.3.1. Number of Line Crossing

Number of line Crossing significantly decreased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to Control (health/untreated) group. Whereas, line crossing significantly increased (P-value <0.05) in (L/C) treated group and in rats treated with (BOE) concentrations of (125,250 and 500 mg/kg) as compared to ROT group. Additionally, there was a significant increased (p-value <0.05) in rats treated with (BOE) concentration of (125 mg/kg) as compared to the (L/C) treated group (Figure 3.12).

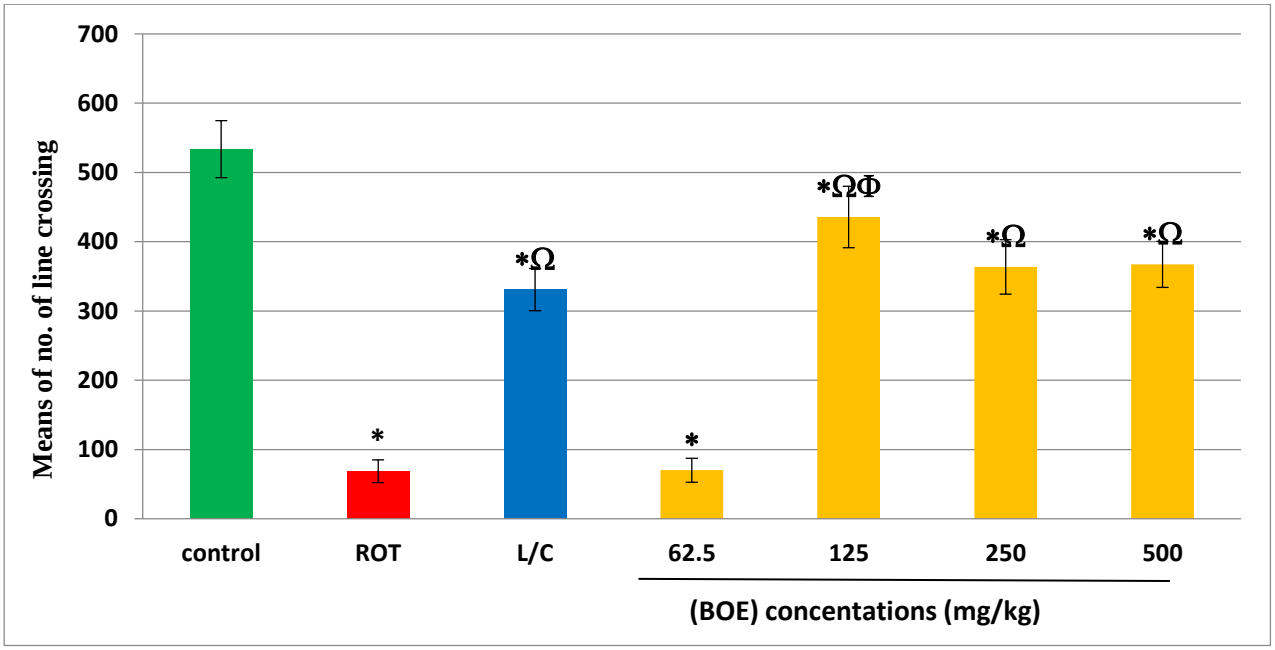

Figure 3.12: Means of number of line crossing ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group .**
Ω=significant increase (p-value <0.05) as compared to the ROT group .
Φ=significant increase (p-value <0.05) as compared to the (L/C) group .

3.2.3.2. Number of Visits to the Center Area

The number of visits to the center area significantly decreased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to control (healthy) group.Whereas, number of visits to the center area significantly increased (P-value <0.05) in (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to ROT group. Furthermore, there was significantly decreased (P-value <0.05) in rats treated with (BOE) concentrations of (62.5 and 250 mg/kg) ,but there was no significant difference in rats treated with (BOE) concentrations of (125 and 500 mg/kg) as compared to (L/C) treated group (Figure 3.13).

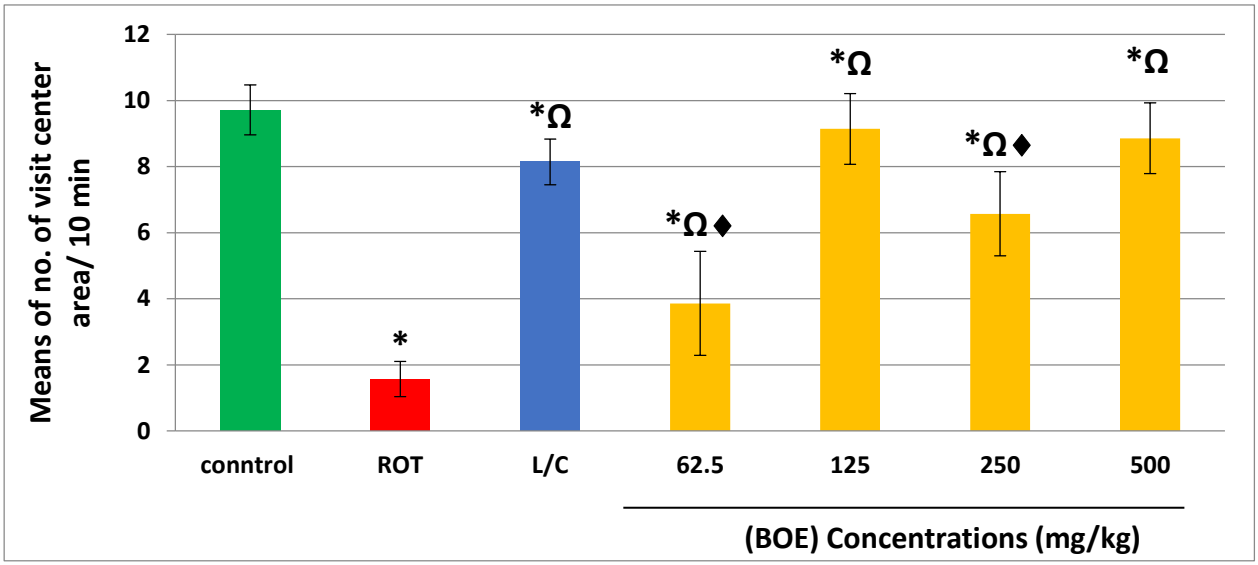

Figure 3.13: Means of number of visit to center area ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
♦=significant decrease (p-value <0.05) as compared to the (L/C) group.

3.2.3.3. Number of Rearing

Number of rearing considerably decreased (P-value <0.05) in rotenone(ROT) group, L-dopa/Carbidopa (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to control (healthy/untreated) group .Whereas, no. of rearing considerably increased (P-value <0.05) in (L/C) treated group and in rats treated with (BOE) concentrations of (125,250 and 500mg/kg) as compared to ROT group .furthermore, it considerably increased in rats treated with (BOE) concentrations of (125 and 250mg/kg) as compared to (L/C) treated group (Figure 3.14).

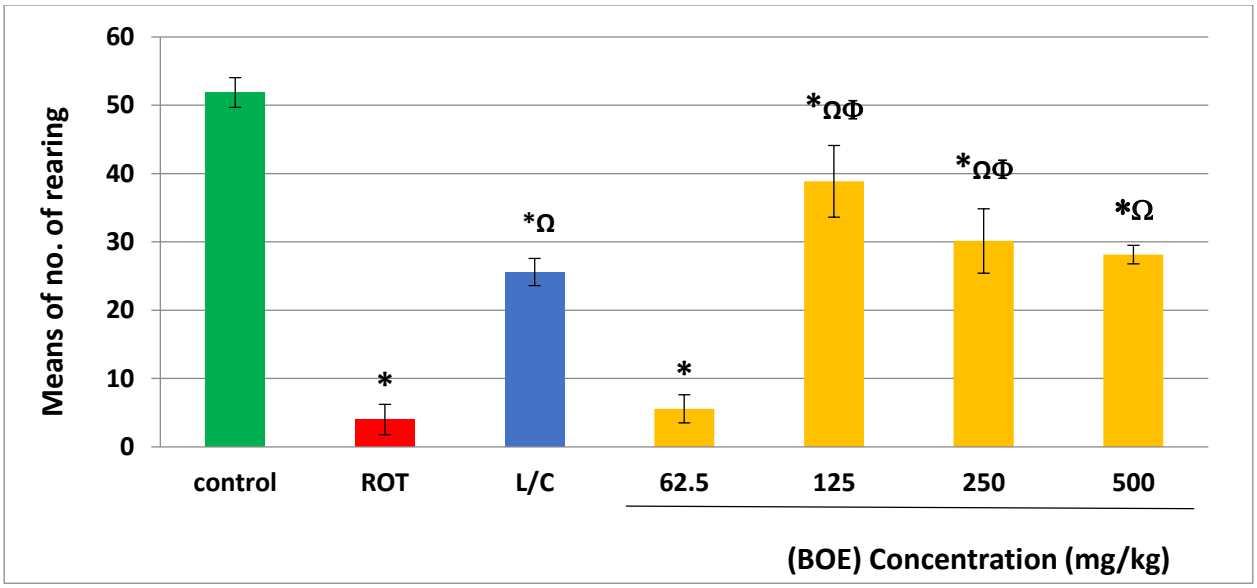

Figure 3.14: Means of number of erect postures (rearing) ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg)

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group .

3.2.3.4. Grooming Time

Grooming time has significantly decreased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500mg/kg) as compared with control (healthy/untreated) group. There was a significant increased (p-value <0.05) in (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500 mg/kg) as compared to ROT group. Furthermore, grooming time has a significant decreased (p-value <0.05) in rats treated with (BOE) concentrations of (62.5, 250 and 500mg/kg),but there was no significant difference in rats treated with (BOE) concentration of (125mg/kg) as compared to (L/C) treated group (Figure 3.15).

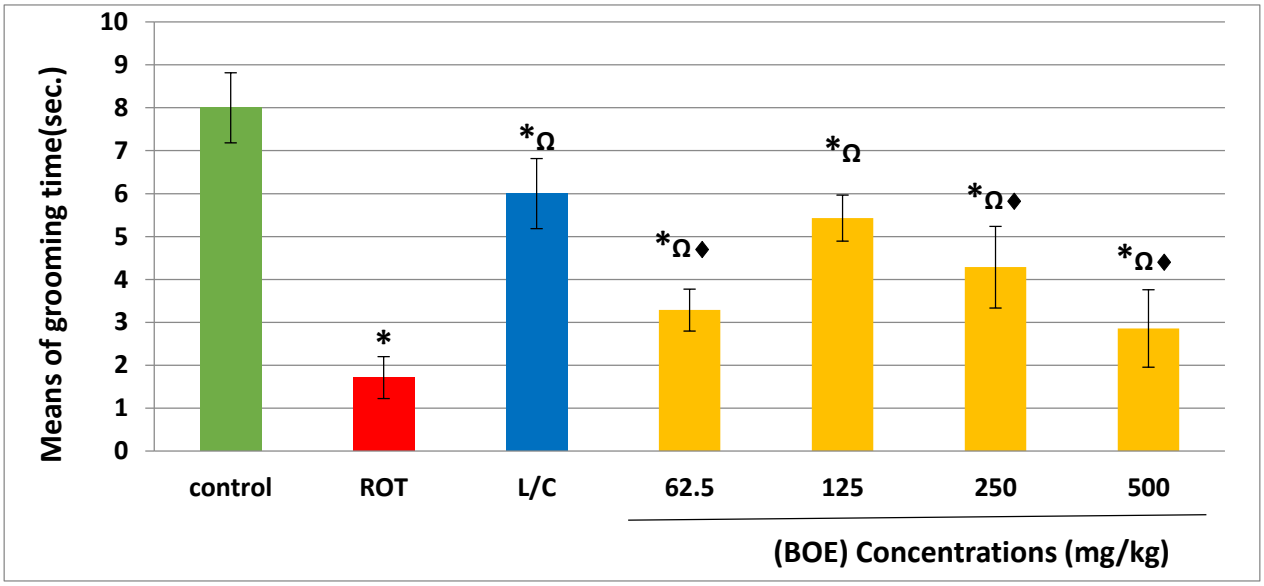

Figure 3.15: Means of grooming time ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group .
♦=significant decrease (p-value <0.05) as compared to the (L/C) group.

3.2.4. The latency of the Falling Time Test (Force Gripping test)

The falling time latency considerably decreased (p-value<0.05) in rotenone (ROT) group and in rats treated with (BOE) concentrations of (62.5,250 and 500mg/kg), but there was no significant difference in rats treated with (BOE) concentration of (125mg/kg) and L-dopa/Carbidopa (L/C) treated group as compared with control (healthy) group. Whereas, there was a significant increased (p-value <0.05) in (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,125,250 and 500mg/kg) as compared to ROT group. Furthermore, there was a significant decreased (p-value <0.05) in rats treated with (BOE) concentrations of (62.5 ,250 and 500mg/kg) and insignificant difference in rats treated with (BOE) concentration of 125mg/kg as compared to (L/C) treated group (Figure 3.16).

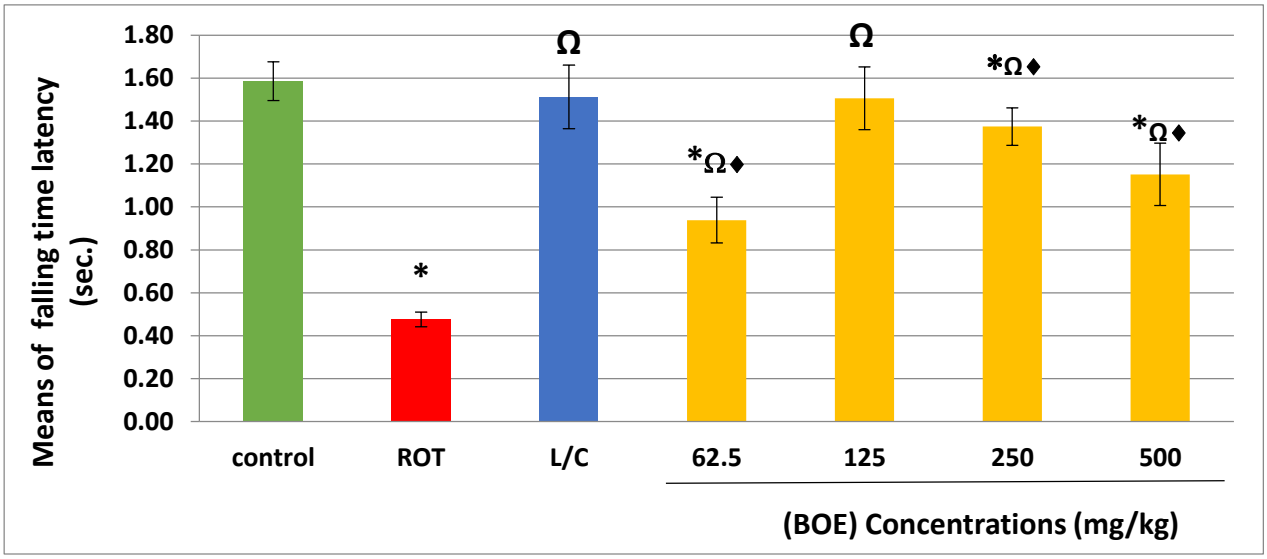

Figure 3.16: Means of falling time latency ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group.
♦=significant decrease (p-value <0.05) as compared to the (L/C) group.

3.2.5. Biochemical Study

3.2.5.1. Malondialdehyde (MDA) Levels

MDA levels significantly increased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) group and in rats treated with *B.oficinalis* extract (BOE) concentrations of (62.5,125,250 and 500mg/kg) as compared to control (healthy) group. whereas, MDA level significantly decreased (p-value <0.05) in rats treated with(L/C) and (BOE) concentrations of (62.5,125,250 and 500mg/kg) as compared with ROT group. Furthermore, there was a significant increased (p-value <0.05) in rats treated with (BOE) concentrations of (62.5,250 and 500 mg/kg) and no significant difference in rat treated with (BOE) concentration of 125mg/kg as compared to (L/C) group (Figure 3.17).

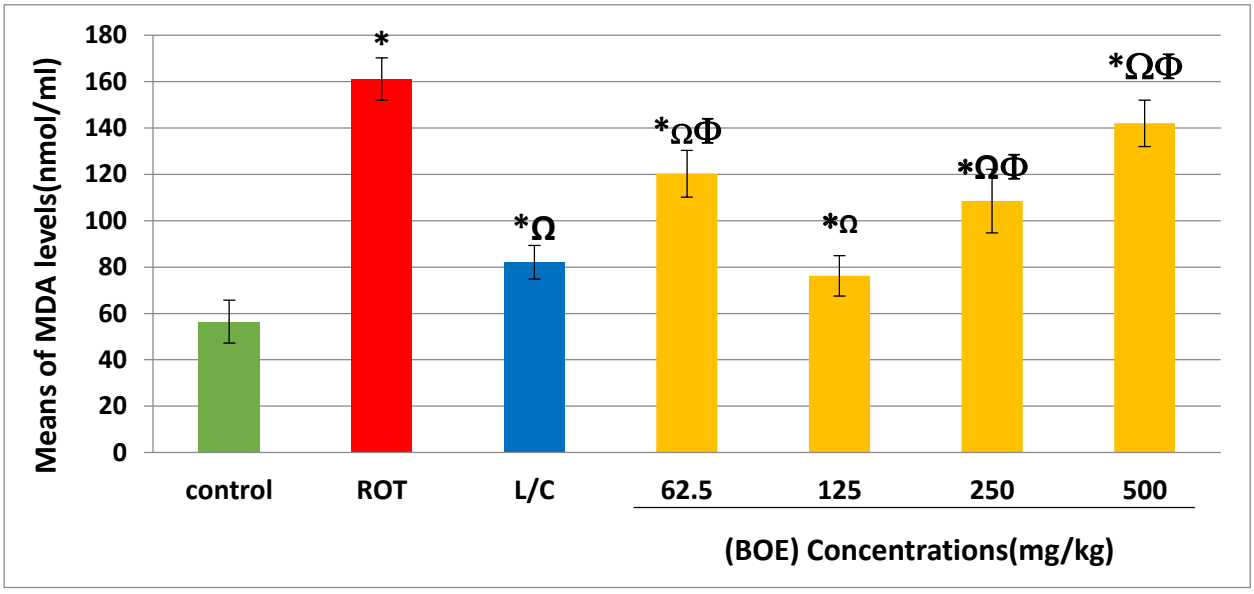

Figure 3.17: Means of MDA levels ± SEM for all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant increase (p-value <0.05) as compared to the control group.**
Ω=significant decrease (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.

3.2.5.2. Interleukin -1Beta (IL-1β) Levels

Interleukin -1β levels significantly increased (p-value <0.05) in rotenone (ROT) group, and in rats treated with (BOE) concentrations of (62.5,250 and 500mg/kg), but there was no significant difference in rats treated with L-dopa/Carbidopa (L/C) and (BOE) concentration of 125mg/kg as compared to control (healthy) group. Whereas,IL-1β significantly decreased (p-value <0.05) in (L/C) treated group and in rats treated with all (BOE) concentrations as compared with ROT group. Additionally, there was a significant increased (p-value <0.05) in rats treated with (BOE) concentrations of (62.5 ,250 and 500mg/kg), but there was no significant difference in rats treated with (BOE) concentration of 125mg/kg as compared with (L/C) group (Figure 3.18).

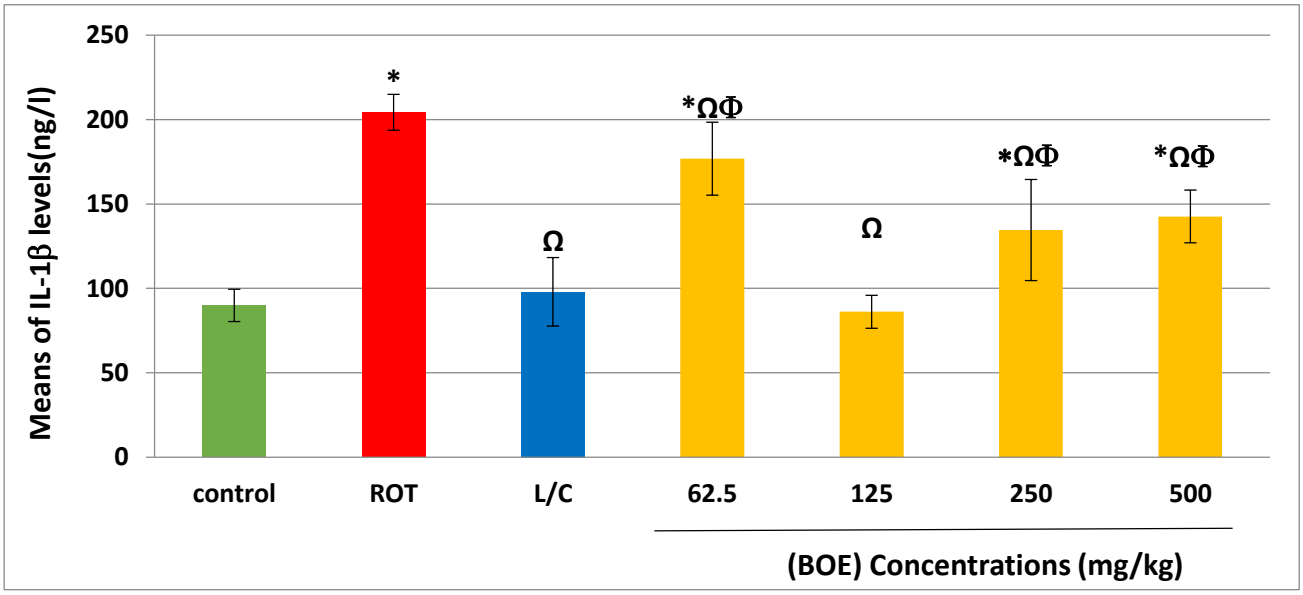

Figure 3.18 : Means of IL-1β levels ± SEM of all groups. Control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant increase (p-value <0.05) as compared to the control group.**
Ω=significant decrease (p-value <0.05) as compared to the ROT group.
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.

3.2.5.3.Total Antioxidant Capacity (TAOC) Levels

Total antioxidant capacity levels significantly decreased (p-value <0.05) in rotenone (ROT) group, L-dopa/Carbidopa (L/C) treated group and in rats treated with (BOE) concentrations of (62.5,250 and 500mg/kg) and there was no significant difference in rats treated with (BOE) concentration of 125 mg/kg as compared to control (healthy) group. Whereas, TAOC significantly increased (p-value <0.05) in (L/C) group and in rats treated with all (BOE) concentrations as compared to ROT group . Furthermore, TAOC significantly increased (p-value <0.05) in rats treated with (BOE) concentration of 125 mg/kg and no significant difference in (BOE) at concentrations (62.5,250 and500 mg/kg) as compared to (L/C) group (Figure 3.19).

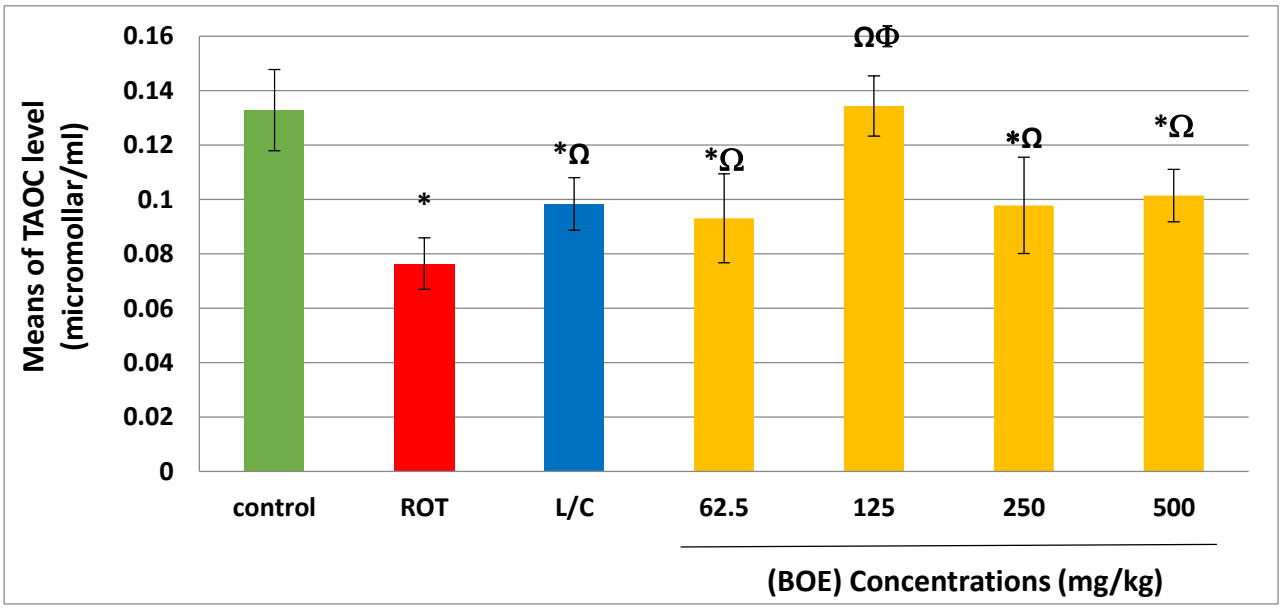

Figure 3.19: Means of TAOC levels ± SEM of all groups. control: healthy untreated group, ROT: (2.5 mg/kg), (L/C): (10mg/kg).

***=significant decrease (p-value <0.05) as compared to the control group.**
Ω=significant increase (p-value <0.05) as compared to the ROT group .
Φ=significant increase (p-value <0.05) as compared to the (L/C) group.

4. Discussion

Parkinson's disease (PD) has many pathologic characteristics such as altered protein homeostasis, mitochondrial dysfunction, and neuroinflammation. The basic motor symptoms of PD involving resting tremor, bradykinesia, muscle rigidity, and postural instability (Jankovic and Tan, 2020).The risk factors for PD include environment, medications, pesticides, ageing, localized cerebrovascular injury, brain microtrauma, and hereditary abnormalities. However, it is unclear how these risk factors work together to cause the disease (Prasad and Hung, 2020).

In experimental research studies, rotenone (ROT) 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 the characteristic of apoptosis, motor impairment and evidence of PD pathologic hallmark(α-synuclein an intracytoplasmic inclusion) (Farombi et al. 2019). Moreover, ROT leads to increased midbrain lipid peroxidation and decrease antioxidant status (Peshattiwar et al. 2020). For all these reasons ,we used ROT (2.5 mg/kg , Intraperitoneally) in our work.

L-dopa/Carbidopa (L/C) ,it is a classical dopaminergic drug for PD. The co-administration of carbidopa with levodopa allows the use of smaller doses of levodopa and reduce the side effects that result from the peripheral actions of dopamine by decarboxylation of levodopa in the peripheral tissues (Motawi et al. 2020).

Flowers of (BOE) exhibit neuroprotective and antioxidant activity in vitro as well as in vivo. (BOE) is able to decrease reactive oxygen species production and increase antioxidant enzyme activity in neuronal cells (Moliner et al. 2022).

In our work, (BOE) reduced the rise in Malondialdehyde (MDA), interleukin-β (IL-1β) levels as well as increases the antioxidant status in brain and SH-SY5Y cells.

4.1. In Vitro

4.1.1. Cytotoxicity Assay in SH-SY5Y Cells

Results show that ROT at a concentration of (40 and 20 µg/ml) caused a significant decrease in the viability of SH-SY5Y cells, which agrees with the study of (Han *et al.*, 2022).

Human catecholaminergic neuroblastoma cell line SH-SY5Y is frequently employed as an in vitro dopaminergic cell model. The SH-SY5Y cells had a high ROT sensitivity, making them an excellent model for studying rotenone (ROT) neurotoxicity in vitro (Sulthana et al. 2022).

However, there has yet to be any experimental research on *B. Officinalis'* ability to protect against ROT-induced neurotoxicity in SH-SY5Y cells.

Cell viability showed a significant reduce in the ROT group as compared to the control group. ROT exposure induces cells apoptosis by inhibiting mitochondrial respiratory chain complex I (MC-1) and elevating reactive oxygen species (ROS) production, ROS activates Abl protein, which causes accumulation of α-synuclein, further disrupts mitochondrial function and ultimately leads to neuronal cell death. Pretreatment with (BOE) raised the level of cell viability in the ROT-exposed cell, which significantly increased at plant concentration 125 µg /ml as compared with the ROT group. Han et al., 2022 reported rosmarinic Acid (*B.officinalis* compound) not only reduction levels of (α-syn) but also inhibits Abl protein signaling by reducing the intracellular ROS level. Therefore, inhibition of Abl

could possibly increase the adenosine monophosphate activated protein kinase (AMPK) activity and promote cell survival. Activating AMPK has multiple effects in PD model including changing cellular metabolism, promoting autophagy, enhancing mitochondrial quality control, and increasing antioxidant capacity. Furethermore, rosmarinic acid restored the inhibited mitochondrial membrane potential and ATP content as well as suppressed ROT-induced ROS overproduction. For these reason, (BOE) protected SH-SY5Y cells against ROT by restoring mitochondrial membrane potential, cellular ATP content and inhibition ROS overproduction. (Han et al. 2022).

4.1.2.Effect of *B.officinalis* Extracts on Biochemical and Immunological Parameters

4.1.2.1.Malondialdehyde (MDA) Assay in Vitro

The current study revealed an increase in the Malondialdehyde (MDA) level in the ROT group as compared to the control (healthy) group, which agrees with the study of Pan *et al.*, 2020 (Pan et al. 2020). Rotenone, which dysregulate multiple cellular pathways, focusing on mitochondrial dysfunction and oxidative stress. Additionally, impairing mitochondrial respiration and enhancing reactive oxygen species (ROS) production, and inhibit proteasomal activity (Xicoy and Wieringa, 2017).

Cells pretreated with (BOE) at (125,250 and 500 μg/ml),showed a significant reduction in MDA levels as compared to the ROT group. (BOE) exhibited significant superoxide-free radical scavenging activity and ferric ion reducing power capabilities attributed to the sufficient quantity of phenol and flavonoid compounds (Kaempferol and Quercetin), which lowering MDA level (Manthena et al. 2022) . Pan *et al.,* 2020 reported Kaempferol increased catalase (CAT),

superoxide dismutase (SOD), Glutathione peroxidase (Gpx), and Glutathione (GSH) levels and decreased the level of lipid peroxidation, indicating its antioxidative potential, which might explain its neuroprotective effect (Pan et al. 2020). Quercetin (QCT), a natural flavonoid, could protect against dopaminergic neuron death by inhibiting ferroptosis through activating the Nrf2 protein. Ferroptosis, a novel form of regulated cell death, is caused by accumulation of lipid peroxides and excessive iron deposition (Lin et al. 2022).

In the L-dopa/Carbidopa (L/C) treated group, the MDA level has been increased as compared to the control group but less than the ROT group. Levodopa (L-DOPA) significantly reduced the levels of ROS and is indicative of the protective roles of the compounds against oxidative stress damage (Kesh et al. 2021). However, long-term L-dopa medication might increase dopaminergic neuronal cell death and precipitate progression of disease, L-dopa might operate as a source of free radicals that limit the activity of mitochondrial enzymes (Shin et al. 2009).

4.1.2.2. Interleukin -1β (IL-1β) Assay in Vitro

According to the current work results, the rotenone (ROT) group significantly increased of IL-1β compared to the control (healthy) group.

Rotenone exposure causes cell death might be due to reduction in ATP production, through the inhibition of complex-I of the mitochondrial respiratory chain and an increase in ROS generation (Xicoy and Wieringa, 2017).

In the current work, we assessed the neuroprotective effects of (BOE) in the SH-SY5Y model under ROT stress. By inhibiting ROS formation and functioning as an antioxidant, (BOE) greatly reduced the cell apoptosis that ROT induced in SH-SY5Y cells (Han et al. 2022).This might be due to the antioxidant components

in (BOE) have been activated and included non-enzymatic antioxidants like Vitamin C and enzymatic antioxidants like Catalase, which maintain the membranes of live cells (Gazwi *et al.*, 2018). Additionally, flavonoids ,Sinapic acid and chlorogenic acid components of (BOE) might be control how several cytokines are expressed and activated including IL-1β, TNF-α, IL-6, and IL-8.Also, these substances have demonstrated the capacity to control the expression of certain genes associated with inflammation and decrease the activity of pro-inflammatory enzymes like iNos and COX-2 (Michalak and Zag, 2023). sinapic acid down-regulated the expression of genes PARKIN1, caspase-3, and DJ-1, along with a reduction in the expression of inflammatory markers such as IL-1β and TNF-α. Further, increase the levels of BCl2, an anti-apoptotic protein, and the activity of superoxide dismutase (SOD), an enzymatic antioxidant (Prabhakar and Ahmed, 2023).

In the L/C treated group, there was a significant decreased in IL-1β as compared to the ROT group. An established L-dopa action mechanism is the down regulation of ROS in biological systems (Edobor et al. 2021).

4.1.2.3. Total Antioxidant Capacity Assay in Vitro

Rotenone (ROT) group significantly decreased in the total antioxidant capacity (TAOC) levels as compared to the control (healthy) group, which agrees with the previous study by Rahimmi et al. 2022 (Rahimmi et al. 2022). Han et al., 2022 reported that rotenone induced toxicity and ROS generation in SH-SY5Y cells leaded to mitochondrial dysfunction by the changes in mitochondrial transmembrane potential. The disruption of mitochondrial complex-I activity and mitochondrial transmembrane potential leaded to the reduction of ATP production (Han *et al.*, 2022).

After (BOE) treatment, a significant increase in TAOC levels is produced in Parkinson's affected at (125 and 500μg/ml) in comparison to the ROT group. Parikh 2019 reported (BOE) flower exhibited the high total phenol (Rosmarinic, gallic and Caffeic acids) and flavonoids contents. Rosmarinic acid has been decreased the formation of nitric oxide (NO) and prostaglandin-E2 (PGE2) and the activity of Cyclooxygenase-2(COX-2) and Inducible nitric oxide synthase (iNos). Gallic acid lowered the ratio of the pro-apoptotic Bax protein and the anti-apoptotic Bcl-2 protein in SH-SY5Y cells. Bcl-2 protein is considered to be an anti-apoptotic protein which inhibits the release of cytochrome c. In contrast, Bax is a pro-apoptotic protein elevates the programmed cell death through affecting the membrane permeability. Additionally, gallic acid elevated the anti-oxidant enzymes such as SOD, CAT and GPx. (Chandrasekhar *et al.*, 2018).

The L/C treated group has been increased in TAOC as compared to the ROT-treated group. Levodopa significantly decreased ROS levels, indicating that the substances had oxidative stress damage prevention functions. Additionally, it markedly reduced the levels of the oxidative stress markers CAT, GSH, and SOD enzymes (Kesh et al. 2021).

4.2.In Vivo

4.2.1.Effect of *B.officinalis* Extracts on Weights of Rats

In comparing rotenone (ROT) group with control group (not treated and not exposed to ROT), it has been revealed a significant decrease in the rat's weight after 21 days. Rats weight have been assessed prior to treatment and every ten days. Throughout the study, the average weight of the rats in the control group have been grown. On the other hand, Rats in the ROT group had lost weight, which was thought to be linked to gastrointestinal neuron damage (Drolet et al.

2009). Furthermore, ROT leaded to lose great strength and difficulties in moving and eating in rats. 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, Moloney, and Dowd 2006).

After (BOE) therapy, the weight on days 10 and 21 were higher than that in the ROT group and did not significantly change from day 0 to either day 10 or day 21.Our explanations for the unaffected weight, might be due to the effect of (BOE) in reduce blood cholesterol, phospholipids, and triglyceride levels (Yaghmour et al. 2021). Another explanation was introduces by Gazwi and Mahmoud in their study on (BOE) is that might have a gastroprotective effect due to the presence of glycosides phenolics, flavonoids, and tannins in the ethanolic extracts of Borago which it's leading to inhibiting lipid peroxidation and enhancing enzymatic antioxidant defenses (Gazwi *et al.*, 2018).

In L/C group, there was no significant decrease in the weight of day 10 and 21 as compared with day 0. This insignificant decrease in weight might be due to the effect of ROT which is overcome by L-dopa that compensates the diminished DA levels.

4.2.2.Effect of *B.officinalis* Extracts on the Behavioral Tests

4.2.2.1. Rotarod Test

Rotarod apparatus has been used to compare between groups .It has been shown that repeated exposure to rotenone (ROT) substantially decreased muscular coordination (number of rotations, the distance between rotations, and duration between rotations) . These results were compatible with previous studies in which,

the administration of ROT-induced muscular rigidity, loss of muscle control, and reduced body movement (Al-Abbasi et al. 2022) (Aslam et al. 2021).

(BOE) administration enhanced rotation number, rotation distance, and rotation time while maintaining muscular coordination in the rotarod apparatus. This demonstrates that (BOE) positively alleviated Parkinson disease (PD) symptoms in rats, particularly in the rats which received 125 mg/kg of plant. Moreover, Both vitro and vivo tests showed *B.officinalis* to has neuroprotective and antioxidant properties. The total antioxidant activity has been increased, and the extract decreased the reactive oxygen species (ROS) production, these results have been documented in the study of (Moliner *et al.*, 2022) . Furthermore, Phenolic compounds, especially (rosmarinic acid, synergic acid, synaptic acid, rutin, and chlorogenic acid) are also found in borage and contribute to the antioxidant properties (Parikh et al. 2019).

Compared to animals treated with ROT, the L-dopa/Carbidopa group was significantly improved the rotarod performance on day 21 due to the standard levodopa (l-dopa) and carbidopa administration. This might be because of the increased dopamine level , which agrees with the study of (Peshattiwar et al. 2020).

Levodopa has been proved to be the most efficient pharmaceutical treatment for symptoms of PD, increasing lifespan and quality of life (Shin et al. 2009).

4.2.2.2.Open Field and the Latency of Falling Time Tests

Line crossing, number of visits to center area, rearing and grooming decreased significantly in rotenone (ROT) group compared to the other six groups.

Exposure to ROT significantly reduced locomotors activity compared with rats treated with (BOE) at concentrations (125,250,500 mg/kg) respectively.

Rotenone is established to induce significant neuromuscular dysfunction and motor disability in rats which also demonstrated a shorter time to hang on a beam with less tenacity (the latency of falling time test). These findings were similar to that of Farombi in his study (Farombi et al. 2019).

Rats with ROT exhibit motor impairments as a result of dopaminergic neuronal death in the substantia nigra area and decreased striatal dopamine levels. The nigrostriatal system dopamine insufficiency brings on movement problems (Wang *et al.,* 2020).The repeated exposure to ROT led to a serious decline in striatal dopamine levels and significant reductions in muscle coordination during the rotarod apparatus and locomotors activity during the open field test (Issa et al. 2020).

On the other hand, (BOE) therapy sustained the behavioral deficiency with an indication of function recovery with increases in no. of line crossing, no. of rearing, no. of grooming, hanging time and decreased rigidity. In (BOE) treated concentrations (62.5,125,250,500 mg/kg) respectively, it improved the motor coordination of Parkinsonian rats. (BOE) flowers contain chlorogenic acid ameliorated rotenone-induced dopaminergic nerve degeneration and alpha-synuclein accumulation in substantia nigra and augmented mean density of dopaminergic nerve fibers in striatum. Therefore, chlorogenic acid prevented rotenone-induced motor and cognitive impairments and significantly restored the rotenone-induced oxidative stress (Sharma et al. 2022).

L-dopa treated rats demonstrated a significant increase in line crossing, risk factor, rearing, grooming and hanging time as compared with ROT group; these

findings were agree the studies from (Maniyath *et al.*, 2017). Treatment with L-dopa enhanced dopamine and norepinephrine levels, as well as their metabolites and their production and release. As a result, L-dopa is the main therapy for compensating for decreased dopamine levels in PD (Shehata et al. 2020).

4.2.3. Effect of *B.officinalis* on Biochemical and Immunological Parameters

4.2.3.1. Malondialdehyde Levels

The current study revealed that the MDA level in the rotenone (ROT) group significantly increased compared to the control(healthy) group, these finding were consistent with the findings of (Wang et al. 2020).

Preclinical studies clearly showed that environmental variables such as neurotoxins, insecticides, pesticides, and dopamine induce oxidative stress in PD. Oxidative stress has been associated with the development of PD in both preclinical and clinical investigations particularly elevation in the concentration of oxidative markers such MDA. Pesticides like ROT have been proven to increase ROS by inhibiting mitochondrial complex-1(MC-I) function. This leads to oxidative stress, which might be the cause of α-synuclein accumulation (Parkhe et al. 2020).

On the other hand, there was a significant decreased in MDA levels in PD-affected rats groups which were treated with (BOE) at concentrations (125,250,500 mg/kg) respectively. The flowers of *B.Officinalis* are an excellent source of polyunsaturated fatty acids (PUFA), flavonoid (kaempferol) and several polyphenols (caffeic, rosmarinic, and chlorogenic acid) which play a significant role in reducing a wide range of free radicals , ROS ,and preventing lipid

peroxidation (Parikh et al. 2019). The mechanism by which PUFA reduces lipid peroxidation appears to include detoxifying peroxy radicals (Mbiydzenyuy *et al.,* 2018). Furthermore, Kaempferol increased catalase, super oxide dismutase (SOD) and glutathione (GSH) levels and decreased the level of lipid peroxidation, indicating its anti-oxidative potential, which may explain its neuroprotective effect (Pan *et al.,* 2020). Chlorogenic acid exerted its neuroprotective effect against rotenone-induced oxidative stress by restoring the glutathione levels and decreasing malondialdehyde content in both striatum and cortex (Sharma et al. 2022). Therefore, an oxidative stress inhibitor could be one of the mechanisms of the antiparkinson effects of *B. officinalis.*

MDA levels significantly increased in the L-dopa/Carbidopa treated group as compared to the control group, but less than the ROT group, which agrees with the study (Motawi et al. 2020). Treatment with L-dopa caused oxidative stress by significantly lowering glutathione levels and raising MDA levels and oxidized glutathione. This finding could be read as evidence, that repeated L-dopa administration increases the production of dopamine, which could then result in an excessive free radical generation that overwhelms the body's natural defences and causes an excess of oxidative stress (Shehata et al. 2020).

4.2.3.2.Interleukin -1β Levels

According to our study, the IL-1β level increased significantly in the rotenone (ROT) group compared to the control (healthy) group, which agrees with the previous study by (Sharma and Raj, 2020). This increase confirm that ROT activates microglia and astrocytes. Nitric oxide and superoxide are free radicals released by activated microglia, and pro-inflammatory marker expression in rats like TNF-α, IL-6, and IL-1β might be increased and triggering various

inflammatory cascades. These inflammatory cascades reduce neuronal function, demonstrating the significance of neuroinflammation in neurological conditions like Parkinson's disease (Sharma and Raj, 2020).

After (BOE) treatment, IL-1β levels in the Parkinson's-affected rats significantly decreased at all (BOE) treated concentrations as compared with the ROT group. Additionally, there was a significant reduction in IL-1β at plant treated concentration 125mg/kg as compared with the control group. Gamma Linolenic Acid (GLA) is a crucial component of (BOE) and has been shown through experimentation to lower the production of IL-1β, which may contribute to inflammation .GLA has shown to reduce the effects of pro-inflammatory cytokines and pathways like tumor necrosis factor-α (TNF-α), nuclear factor kappa light chain (NF-κB), nitric oxide (NO) production from inducible nitric oxide synthase (iNOS), and production of prostaglandin PG. NF-κB may be responsible for the activation of many inflammatory cytokines and enzymes like iNOS and cyclooxygenase (COX) and activation of many pathways linked with oxidative stress, the decrease in the activity of NF-κB can decrease the production of NO, TNF-α and ROS, thus decreasing the levels of inflammation and oxidative stress in the body (Parikh et al. 2019).

In the L/C treated group, IL-1β significantly decreased as compared with the ROT group ,which agrees with a previous study (Chen et al. 2021).

4.2.3.3.Total Antioxidant Capacity (TAOC) Levels

TAOC levels significantly decreased in ROT group as compared with the control (healthy) group, which is consistent with the earlier study's findings (Mbiydzenyuy et al. 2018).

Dopaminergic neuronal death in the brains of PD patients might be caused by excessive ROS generation in the brain causes more oxidative stress, metabolism of dopamine increased oxidative stress leaded to mitochondrial dysfunction, elevated iron and calcium levels in substantial nigra, and neuroinflammation (Chang and Chen 2020).The mitochondria are a major generator of free radicals. This is because mitochondrial organelles are responsible for more than 90% of cellular oxygen consumption, and oxygen metabolism always generates radicals in biological systems. On the other hand, Cells have evolved enzyme machinery to resist oxidative stress throughout time. Superoxide dismutase (SOD), for example, is an enzyme that catalyzes the transformation of 02 - to H2O2 and O2. It is present in the matrix of mitochondria, which is where oxidative phosphorylation takes place. Catalase (CAT) and glutathione peroxidase (GPXs) are two more antioxidant enzymes that aid the conversion of H2O2 to molecular oxygen and water, lowering the most common reasons of oxidative stress. All of these enzymes have been demonstrated to protect neurons against oxidative stress (Kumar et al. 2012).

After (BOE) treatment, a significant increase in TAOC levels was noticed in rats affected by Parkinson's at concentrations (62.5,125,250 and 500 mg/kg) when compared with the ROT group, especially at plant treated concentration 125mg/kg, which shows a significant increase in TAOC when compared with other treated concentrations of plant. *B.officinalis* flower exhibited the highest total phenolic and flavonoid contents, especially chlorogenic, caffeic acids and quercetin. Caffeic acid is believed to remove excess reactive oxygen species /reactive nitrogen species generation, and is known to stimulate antioxidative enzyme activities including SOD, GPx and CAT (Balakrishnan et al. 2021). Quercetin, was capable of decreasing the oxidative load on neuronal cells in the

striatum as evidenced by increase in total GSH and SOD levels (Haleagrahara, Siew, and Ponnusamy 2013).

The (L/C) treated group demonstrated a significant increased in TAOC and in antioxidant marker activity compared to the ROT-treated group. The finding backs up the earlier investigation of Mbiydzenyuy *et al.*, 2018 which evaluated the antioxidant activity of L-Dopa and carbidopa in PD and its implications for oxidative stress. Moreover, the study of (Mbiydzenyuy *et al.*, 2018) had also shown that L-dopa acted as a DNA protector, a free radical scavenger, and a gene regulator in cellular oxidative metabolism. Although there were not enough human studies to conclusively show whether L-dopa has protective or harmful effects on the life of DA-ergic cells in PD patients, the combined therapy (carbidopa plus L-dopa) is effective in both lengthening the L-Dopa t1/2 and improving the redox status of PD patients. Moreover, inducing the expression of anti-oxidant enzymes taken part in anti-oxidant induction by improving the expression of CAT and GPX, despite of the truth that the oxidation of L-Dopa and dopamine might produce ROS. However, Carbidopa's protective activity is attenuated, and it becomes more pro-oxidant, like many other antioxidant compounds (Colamartino *et al.*, 2014).

Finally, the findings of this study showed that (BOE) dose of 125mg/Kg had a strong improving effect on behavioral tests and biochemical tests in comparison with other doses of plant which showed less efficacy in attenuating the symptoms of parkinsonism.

Conclusions

In Vitro

1. Ethanolic extract of *B.officinalis* effectively decreases the level of IL-1β in SH-SY5Y cells, which might play an important role in attenuating the progression of Parkinson's disease.
2. Treatment with (BOE), effectively decreases the level of MDA, the highly toxic molecule in SH-SY5Y cells, indicating its effective role as an anti-oxidant agent and as a defensive agent protecting the tissue from the effect of excessive free radicals which is one of the causes of Parkinson's disease.

In Vivo

1.Ethanolic extract of *B. officinalis* at 125 mg/kg attenuates the neuroscaler impact in male rats produced by ROT administration.

2. Ethanolic extract of *B. officinalis* effectively decreases the levels of IL-1β in brain tissues, which might play an important role in attenuating the progression of Parkinson's disease.

3.Treatment with (BOE) in rats , effectively decreases the level of MDA, the highly toxic molecule, in brain tissues , indicating its effective role as an anti-oxidant agent and as a defensive agent protecting the tissue from the effect of excessive free radicals which is one of the causes of PD.

Recommendations

1. Study the effectiveness of *B.officinalis* extract (BOE) in protecting or improving the signs of Parkinson's disease using the plant extract in combination with other anti-Parkinson drugs.
2. Study the biological effects of separated and purified active constituents of (BOE) in protecting and treating PD signs.
3. Molecular studies, which include the mRNA expression and immunoblot analysis, may be conducted to evaluate the anti-parkinson effect of *B. officinalis*.
4. Clinical trials are needed to study the effect of *B. officinalis* extract on patients with PD.

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الخلاصة

مرض الشلل الرعاش هو مرض التنكس العصبي الأكثر شيوعا بعد مرض الزهايمر ، ويتميز بالحركة والاهتزاز الذي لا يمكن السيطرة عليه والتقلب وعدم الاستقرار الوضعي والرعشة.

العمليتان الرئيسيتان اللتان تسببان مرض الشلل الرعاش هي الإجهاد التأكسدي والالتهاب العصبي اللذان يرتبطان ارتباطا وثيقا بموت الخلايا العصبية الدوبامينية في المنطقة السوداء الكبيرة مما يؤدي إلى استنفاد الدوبامين المميت.

الروتينون هو سم عصبي يدمر الخلايا العصبية الدوبامينية بشكل انتقائي ويسبب أعراضا تشبه مرض الشلل الرعاش في الجرذان ويستخدم لاستحثاث مرض الشلل الرعاش.

كان الهدف من هذه الدراسة هو تقييم تأثير المستخلص الأيثانولي لزهور لسان الثور في نماذج مرض الشلل الرعاش المستحثة بواسطة الروتينون في الجرذان وفي خلايا SH-SY5Y.

في المختبر:

تم زرع وتنمية خطوط خلايا SH-SY5Y في طبق زراعة الأنسجة (96 حفرة) لمدة 24 ساعة ثم تمت معالجة الخلايا بتراكيز مختلفة من المستخلص الأيثانولي لزهور لسان الثور بتخفيفات تسلسلية (31.25-500مايكروجرام/مل) ودواء (ليفودوبا/كاربيدوبا) بتركيز (10مايكروجرام/مل) وتركت مجموعة من الخلايا بغير معالجة كمجموعة مراقبة، وحضنت لمدة ساعتين وبدرجة حرارة 37 درجه سيليزيه ثم تمت معالجه الخلايا بواسطة مادة الروتينون بتركيز(20مايكروجرام/مل) وبعد فترة حضانة مدتها 48 ساعة بعد فترة التعرض تم اخذ رائق الوسط والتي تستخدم في الاختبارات البيوكيميائية لتحديد تراكيز MDA، IL-1β و TAOC اللونية.

تم الحصول على النتائج التالية في المختبر:

المستخلص الأيثانولي لزهور لسان الثور عند تراكيز (500،125 مايكروجرام /مل) تظهر انخفاضا معنويا في مستويات MDA وIL-1β. وزيادة معنوية في مستوى TAOC مقارنة مع مجموعة الخلايا المعالجة بواسطة الروتينون فقط.

في الجسم الحي:

في هذه الدراسة التجريبية التي امتدت على مدى ثلاثة أشهر، تم استخدام حوالي 70 فأراً من ذكور البينو الأصحاء ، مقسمة بالتساوي إلى المجموعات السبع التالية: الأولى هي المجموعة الضابطة السليمة وغير المعالجة، في حين تلقت المجموعات الستة المتبقية مادة الروتينون بتركيز (2.5 ملغم / كغم) داخل الصفاق من يوم لآخر ولمدة 21 يوم، وتلقت المجموعة الثانية مادة الروتينون فقط، بينما تلقت المجموعة الثالثة جرعات فموية قدرها (10ملغم/كغم) من دواء (ليفودوبا/كاربيدوبا) يومياً، والمجموعة الرابعة ،الخامسة، السادسة والسابعة جرعات فموية بتراكيز قدرها (62.5 ،125، 250و 500ملغم / كغم) على التوالي من مستخلص الايثانولي لزهرة لسان الثور يومياً .بعد ذلك تم إجراء تحليلات السلوك العصبي عن طريق اختبارات الروتارود، والحقل المفتوح، واختبارات الإمساك بالقوة في اليوم 22. بعدها تم قطع رؤوس الحيوانات،وإعداد عينات من أنسجة المخ المتجانس، والذي بعدها يستخدم في الاختبارات البيوكيميائية لتحديد تراكيز MDA، IL-1β و TAOC اللونية.

تم الحصول على النتائج التالية في الفئران:

(1) أظهرت نتائج قدرة جهاز الروتارود على التنسيق بين عضلات الجرذان زيادة معنوية في عدد الدوران ومسافة الدوران وزمن الدوران في الجرعات العلاجية للمستخلص الأيثانولي لزهور لسان الثور بتراكيز (250،125 و 500 ملغم/كغم)، مقارنة مع مجموعة الجرذان المعالجة بمادة الروتينون فقط.

(2) مستخلص زهور لسان الثور بتراكيز (250،125 و 500 ملغم / كغم) حسنت الحركة المنسقة للفئران المصابة بمرض الشلل الرعاشي مع ارتفاعات كبيرة في مسافة العبور وعدد التربية ووقت الاستمالة ووقت التعليق والحد الأدنى من الصلابة.

(3) في الجرذان المعالجة بـالمستخلص الايثانولي لزهور لسان الثور بتراكيز (250،125و500 ملغم/كغم)، أظهرت انخفاضاً معنوياً (قيمة $p < 0.05$) في مستويات MDA وIL-1β وزيادة معنوية (قيمة $p < 0.05$)) في مستويات TAOC مقارنة بمجموعة الجرذان المعالجة بمادة الروتينون فقط .

الخلاصة

يشير هذا إلى أن المستخلص الأيثانولي لزهور لسان الثور يخفف بشكل فعال أعراض مرض الشلل الرعاشي في الجرذان، خاصة عند التركيز 125 ملجم/كجم.

المستخلص الأيثانولي يحتوي على نشاط مضاد لاعراض الشلل الرعاش والذي يمكنه عكس المرض الناجم عن الروتينون في المختبر وفي الجسم الحي. هذه الأنشطة تنطوي على خصائص مضادة للالتهابات ومضادات الأكسدة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/كلية الطب

دراسة التأثير الوقائي العصبي للمستخلص الإيثانولي لزهور لسان الثور على النماذج الحيوانية لمرض الشلل الرعاشي وخط الخلايا SH –SY5Y

رسالة مقدمة إلى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في الأدوية/الأدوية والسموم

من قبل:

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