EVALUATION OF NEUROPROTECTIVE EFFECT OF *Barleria prionitis* Linn IN ANIMAL MODELS OF PARKINSON'S DISEASE

A Dissertation submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-600 032

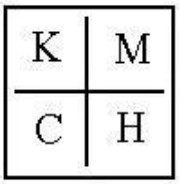
In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

OCTOBER-2017



DEPARTMENT OF PHARMACOLOGY

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Date: **Signature of the guide**

Place: Coimbatore

DECLARATION

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Examination Center: KMCH College of Pharmacy, Coimbatore

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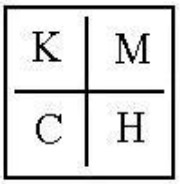

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Dedicated to Almighty,

My Beloved Parents, Sister

and Friends

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TABLE OF CONTENTS

SL: NO	CONTENTS	PAGE NO
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	9
3	METHODOLOGY	32
4	RESULTS	49
5	DISCUSSION	97
6	CONCLUSION	104
7	BIBLIOGRAPHY	105

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
%	Percentage
AEBP	Aqueous extract of barleria prionitis
ATP	Adenosine tri phosphate
BBB	Blood brain barrier
CMC	Carboxy methyl cellulose
COMT	Catechol-O-Methyl Transferase
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals
DA	Dopamine
DAG	Diacylglycerol
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-dihydroxy phenyl acetic acid
DMSO	Dimethyl sulfoxide
DTNP	5,5'-dithiobis-2-nitrobenzoic acid
G	Gram
GABA	Gamma Aminobutyric Acid
GSH	Reduced glutathione
5-HT	5- Hydroxytryptamine
IAEC	Institutional Animal Ethical Committee
i.p	Intraperitonial
L-dopa	Levodopa
LPO	Lipid peroxidation
LRRK 2	Leucine rich repeat kinase 2
MAO-A	Monoamine Oxidases Type-A
MAO-B	Monoamine Oxidases Type-B
MAOIs	Monoamine Oxidases Inhibitors
Mg/kg	Milligram/kilogram
$MPDP^+$	1-methyl-4-phenyl-2,3-dihydropyridinium ion
MPP^+	1-methyl-4-phenyl-pyridinium ion
MPTP	1-Methyl-4-Phenyl-1, 2, 3, 6-Tetra-Hydropyridine

ng/g	Nanogram per gram
nm	Nanometer
OECD	Organisation for Economic Corporation and Development
PBS	Phosphate buffer solution
p. o	Post Oral
PCD	Programmed cell death
PD	Parkinson ’s disease
REM	Rapid eye movement
ROS	Reactive oxygen species
rpm	Rotations Per Minute
SD	Standard deviation
sec	second
SEM	Standard Error Mean
SNpc	Substantia nigra par compacta
TBA	Thio barbituric acid
TBARS	Thio barbituric acid reactive species
TCA	Trichloro acetic acid
UCH-L1	Ubiquitin C-terminal hydrolase L1
µg/mg	Microgram per milligram
w/v	Weight per volume

ABSTRACT

The present investigation has been undertaken as study the anti-parkinson activity of aqueous extract of *Barleria prionitis*. The plant *Barleria prionitis* of family *acanthaceae* an ayurvedic herb which is known for its significant medical properties. Experiments were conducted following standard procedures. The aqueous extract of *Barleria prionitis* were evaluated for their *invivo* antioxidant and anti-parkison properties and neurotransmitters level. The anti-parkinson activity of AEBP was evaluated using MPTP induced parkinson and Rotenone induced Parkinson models. Levodopa was used as standard for both models. Extracts treated groups showed higher *invivo* antioxidant and anti-parkinson activities. They also showed higher activity in neurotransmitters level. AEBP exhibited similar anti-parkinson activity that of standard but with lesser magnitude. The result may be attributed to the chemical constituents such as iridoid glycosides present in it which may be due to their individual or cumulative effect that enhanced anti-parkinson activity and provided scientific evidence of the ethnomedicinal futures of *Barleria prionitis*. These findings could justify the inclusion of this plant in the management of parkinson's disease.

Keywords: AEBP, anti-parkinson, MPTP, rotenone, chemical constituents.

1. INTRODUCTION

1.1 INTRODUCTION

This chapter present: back ground of study, statement of the problem, definition of terms, theoretical framework, purpose of the study, hypothus and specific aim.

1.1.1 BACK GROUND OF STUDY

Parkinson Disease (PD) is the second most chronic neurodegenerative disorder in the world, after Alzheimer´s Disease (AD), and is estimated to affect about 2% of the population over 60 years of age. The disease generally affects persons aged 55–64 years, although occasionally much younger individuals are affected. Although the causes for degeneration of dopaminergic neurons in Parkinson's disease are not well understood, The degeneration of these dopaminergic neurons leads to four cardinal, debilitating symptoms: resting tremor, muscular rigidity, bradykinesia, and postural imbalance.[1,2]

At current research, the etiology of PD is still not clearly known. Evidences suggest massive oxidative stress leading to the formation of free radical. Pathologically the hallmark of Parkinson disease are the severe loss of dopaminergic neuron in the substantia nigra pars compacta and the presence of proteinaceous inclusion called lewy bodies, which is mainly composed of fibrillar α-synuclein and ubiquitinated protein with in some remaining nigral neurons.[3,4]

The plant Barleria prionitis contains Iridoids, Barlerin, Acetyl barlerin, β-sitosterol, Prioniside, Barlerinoside, verbascoside, 7-methoxy diderroside, Anthroquinones, Flavonoids. The plant used for Stomach disorder, Urinary infections, fever, Tooth ache, Diuretic, Jaundice, Asthma, Arthritis, Inflammation, Migraine, Dropsy, Leprosy.[5,6]

1.1.2 STATEMENT OF PROBLEM

In 2015, PD affected 6.2 million people and resulted in about 117,400 deaths globally. Parkinson's disease typically occurs in people over the age of 60, of which about one percent are affected. Males are more often affected than females. When it is seen in people before the age of 50, it is called young-onset PD.[7]

The World Health Organization gives an "estimated crude prevalence" (the total number of existent cases each year, old and new) of 160 per 100,000, and an estimated incidence (the number of new cases each year) of 1619 per 100,000. The incidence of PD varies across the globe. However, this distribution may not be as simple as a geographical or ethnic factor. It is known that the PD is more prevalent in North America and Europe than in Asia and West Africa. However, research has been carried out to know the causes of PD and the use of medicinal plants for its treatment, prevention, and cure.[8]

In China alone, there are more than 1.7 million people with PD. The prevalence of PD per 100,000 of population varies from country to country, with highest in Albania (800) followed by Egypt (557), USA (329107), Israel (256), Japan (19376), Germany (183), Spain (170122), Italy (168104), Finland (166120), Bulgaria (164137), Estonia (152), Australia (146104), England (139121), Portugal (135), Cuba (135), Canada (125), China (11957), Scotland (129), Norway (102), Thailand (95), Sweden (76), New Zealand (76), Nigeria (67), Poland (66), Jordan (59), etc.

The Parsi community in Mumbai has the world's highest incidences of PD where it affects about 328 out of every 100,000 people despite living in a country, India, with one of the world's lowest incidence of PD (70 out of 100,000). The world's highest prevalence of Parkinson's Disease of any country in the world is Albania at a rate of 800 per 100,000. Ethiopia has the world's lowest recorded prevalence of Parkinson's Disease. At a rate of only 7 per 100,000.

There are more men than women with Parkinson's Disease. However, the ratio of males to females who have Parkinson's Disease differs a lot according to the country. In Nigeria there are far more men than women who have Parkinson's Disease. In Japan more women than men have Parkinson's Disease.[9]

1.1.3 DEFINITION OF TERMS

Antioxidants

Substances found in certain foods and supplements that can remove toxic free radicals from the body.[10]

Parkinson's Disease

Parkinson's Disease is the second most common progressive neurodegenerative disorder and Characterized by both motor and non-motor symptoms, PD patients classically display rest tremor, rigidity, bradykinesia, and stooping posture. PD can also be associated with neurobehavioral disorders (depression, anxiety), cognitive impairment (dementia), and autonomic dysfunction (e.g., orthostasis and hyperhidrosis).[11]

Tremor

Tremor, a rhythmic, involuntary, oscillatory movement of body parts, is the most common movement disorder. Tremors are classified as rest or action tremors. Rest tremor occurs when the affected body part is completely supported against gravity. Action tremors are produced by voluntary muscle contraction and are further divided into postural, isometric, or kinetic tremors.[12]

Rigidity: Stiffness of the muscles[13]

Bradykinesia: Slowness of movement; a common motor symptom of PD.[14]

Postural instability

A tendency to fall or the inability to keep oneself from falling; imbalance.[15]

Oxidative stress

Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species (ROS), such as hydrogen peroxide, organic hydro peroxides, nitric oxide, superoxide and hydroxyl radicals etc.[16]

Mitochondrial dysfunction

Mitochondrial dysfunction(s) (MDs) can be defined as alterations in the mitochondria, including mitochondrial uncoupling, mitochondrial depolarization, inhibition of the mitochondrial respiratory chain, mitochondrial network fragmentation, mitochondrial or nuclear DNA mutations and the mitochondrial accumulation of protein aggregates.[17]

Neurotransmitters

Neurotransmitters are chemical messengers which are responsible for communication among neurons. Neurons are the cells of chemical communication in the brain.[18]

Alpha-synuclein

Alpha-synuclein (Syn), a small protein of 140 amino acids specifically enriched in the presynaptic nerve terminals, has been found as a major component of Lewy bodies, with intraneuronal inclusion present in the brain of Parkinson's disease (PD) patients.[19]

Substantia nigra

meaning “black substance” in Latin, a region in the base of the brain that contains dopamine-producing neurons, which appear dark under a microscope; people with PD experience cell loss in this region.[20]

MPTP

(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a prodrug to the neurotoxin MPP+, which causes permanent symptoms of Parkinson's disease by destroying dopaminergic neurons in the substantia nigra of the brain. It has been used to study disease models in various animal studies.[21]

Rotenone

Rotenone is an odorless chemical that is used as a broad-spectrum insecticide, piscicide, and pesticide. It occurs naturally in the roots and stems of several plants. It causes Parkinson's disease if injected into rats.[22]

1.1.4 THEORETICAL BACKGROUND STUDY

Parkinson’s disease (PD) was the first neurological disease and characterized by degeneration of neuromelanin-rich dopaminergic neurons in the substantia nigra pars compacta and the frequent deposition of Lewy bodies. Speech impairment has been reported in 60-80% of the PD patients which reaches up to 100% in the later stages. Lazarus *et al*., subjected PD patients to the Indian Speech and Hearing Association (ISHA) articulation assessment and the Vaghmi software. It was found that 64.7% had slow reading speed, 60.2% hoarseness of voice, 39.8% articulatory defect, and 32.3% jerky speech.

Memory disturbances and dementia are known to occur in later stages of PD. Patients with early PD can have subtle disturbances in neuropsychological testing. To evaluate whether these abnormalities were affected by treatment, PD patients were assessed after 12 weeks of levodopa (LD) therapy.

Several neuropsychiatric manifestations such as depression, anxiety, and sleep disturbances have been described in PD. Depression in PD may be reactive depression as a result of chronic illness as well as a result of neurodegeneration.

Psychosis can also be a part of nonmotor spectrum of PD. It may be disabling to the patients and its presence may also warrant change in treatment strategies. The manifestations include hallucinations and delusions. In a study from India by Amar *et al*., 40 patients of PD with psychosis were assessed. Pure hallucinations were commonest in these patients (85%) and a combination of delusions and hallucinations was found in 7.5%. Of these visual hallucinations were predominant, amounting to 60%.

Animal models are valuable tools for studying the biology and genetics of human parkinson as well as for preclinical investigation of anti-parkinson therapeutics and parkinson preventive studies. Various animal models have been generated by genetic engineering, graft transplantation, and viral/physical/chemical induction. Studies from animal models of parkinson have been utilized for preclinical investigation of therapeutic efficacy and toxicity of chemicals and biologicals. Tremendous advances have been made in the generation of animal models of parkinson, which have become increasingly sophisticated by application of new technologies and integration of clinical information from patients. The goals are to faithfully recapitulate the human parkinson diseases in the animal models and apply them as preclinical tools, with the hope of successfully translating the basic knowledge into treatment and prevention of parkinson in humans.

The mouse has been the traditional animal model for basic and preclinical studies of parkinson, and other organisms including zebrafish play important and complimentary roles as models of parkinson research. Genetically engineered mouse models of parkinson have been generated by a variety of interventions such as chemical or physical mutagenesis, viral infection, insertion of transgenes, homologous recombination, and the recently developed gene edition. There are numerous publications of research studies regarding generation of animal models of parkinson and their pre-clinical applications.[23,24]

MPTP induced PDs

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), is a potent neurotoxin & highly lipophilic. After systemic administration it rapidly crosses the blood-brain barrier, enters astrocytes and is metabolized to its active metabolite MPP+ by monoamine oxidase-B (MAO-B). MPP+ is able to inhibit complex 1 of the mitochondrial electron transport chain, resulting in the formation of ROS & leading to reduced ATP production.

Neuroprotection suggests preventing or slowing disease progression. Nevertheless, despite advances toward this goal, all current treatments are symptomatic; none halt or retard dopaminergic neuron degeneration. L-dopa treatment produces many distressing side effects, and its possible that metabolism of excess dopamine by the monoamine oxidase enzymes in the brain produces too much H2O2. An initial good response to symptomatic pharmacological treatment declines with time, and severe side effects develop and later on surgical interventions are to be used. The progressive neurodegeneration in PD is not arrested by the currently used drug therapies. Hence, recent researches are focusing on finding therapies, preferentially herbal drugs.[25]

ROTENONE induced PDs

PD can be induced with mitochondrial complex I inhibitors such as the environmental toxins rotenone. Rotenone, a commonly used natural pesticide prepared from the roots of tropical plants, such as Derris elliptica, can freely cross cell and mitochondrial membranes. *In vitro*, rotenone has been shown to promote the accumulation and aggregation of alpha-synuclein and ubiquitin, cause oxidative damage, and endoplasmic reticulum stress, and lead to cell death. *In vivo*, chronic exposure of rats to rotenone induces PD-like symptoms, including dopaminergic neurodegeneration and the occurrence of cytoplasmic inclusions similar to Lewy bodies. Re- cent studies show that chronic exposure of Drosophila to rotenone recapitulates key features of Parkinsonism, including selective loss of dopaminergic neurons and locomotor deficits. Although there are also study showing contradictory results, most of the evidences are consistent and suggesting that rotenone exposure contributes to PD-like symptom and that rotenone-based Parkinson's disease models can be used to test potential compounds for PD intervention.[26]

1.1.5 PURPOSE OF THE STUDY

Traditional medicine practitioners have described the therapeutic efficacies of many traditional and indigenous plants against diseases. A large number of plants are used by folklore traditions in India for a treatment of parkinsonism. Various research data revealed that plants may work as anti-parkinson by multiple mechanisms. There are several reports stating that the extracts of several plants, used for parkinson therapy. Natural products that are safe, and possess physiological properties are excellent sources of new therapeutics for the treatment of parkinson. Some researchers, therefore, have shifted their focus to the

potential anti-parkinson properties of plants. Thereby, the search for new agents is ongoing and natural products become a great target. Various literature and studies show that the active principles triterpenoids, iridoids , and flavonoids are having a crucial role in anti-parkinson treatment. *Barleria prionitis* is rich in these types of triterpenoids, Iridoids, and Flavonoids. The purpose of this study is to investigate and evaluate anti-parkinson activity on MPTP and Rotenone-induced model by using aqueous extracts of *Barleria prionitis.*

1.1.6 HYPOTHESIS

I hypothesize that the presence of active constituents like triterpenoids, iridoids and flavonoids in this plant *Barleria prionitis* after isolation and extraction may produce anti-parkinson activity. To test whether the plant producing the anti-parkinson activity, MPTP and Rotenone induced model are selected. The result of these studies will have a translational value to anti-parkinson activity.

1.1.7 SPECIFIC AIMS

1. To determine the in-vivo anti-oxidant capacity of aqueous extract of *Barleria prionitis.*

2. To determine the In-vivo neurotransmitters of aqueous extract of *Barleria prionitis.*

3. To employ the aqueous extract of *Barleria prionitis* having anti-parkinson activity determine by MPTP and Rotenone induced Parkinson model in SD tats and swiss albino mice.

1.1.8 PLAN OF WORK

1. Literature collection

2. Selection, collection and authentication of plant materials

3. Extraction of plant with water by maceration method

4. Preliminary phytochemical analysis

5. Pharmacological study

- ✓ Screening of anti-parkinsonian activity using various behavioural paradigms
- Rota rod
- Actophotometer

- Forced swim test
- Tail suspension test
- Hole board test

✓ In vivo activity

a) Estimation of total protein

b) Antioxidant assays

- Non-enzymatic antioxidant (GSH)
- Estimation of Lipid peroxidation (LPO)

c) Neurotransmitters estimation

- Dopamine, GABA, Serotonin

6. Histopathological study

7. Statistical analysis

2. REVIEW OF LITERATURE

2.1. PARKINSON'S DISEASE

Parkinson's disease (PD) is a chronic, progressive neurologic disease. Affecting one in every 100 persons above the age of 65 years, it is the second most common neurodegenerative disease after Alzheimer's disease. It presents mainly four cardinal motor manifestations such as tremor at resr, rigidity, bradykinesia or slowing of movement and postural instability. The pathological hallmarks of PD are the presence of intracytoplasmic inclusions from protein aggregates called Lewy Bodies (LBs) and the depletion of pigmented dopamine containing neurons in the region known as substantia nigra pars compacta. PD is characterized by the loss of 50-70% of dopaminergic neurons located in the substantia nigra. Thus far, both the cause and the mechanisms of PD remain unknown.[27,28,30]

In about 95% of PD cases there is no apparent genetic linkage which is referred to as sporadic PD, but in remaining cases the disease is inherited. Current evidence suggests an involvement of both environmental and genetic factors in the progression of PD. Researchers on the pathogenesis of PD advanced with the development of animal models. These models have been based on the systemic or local administration of neurotoxins that are able to reproduce pathological and behavioural changes with PD in mammals. Levodopa treatment still remains as the gold standard for PD therapies. Unfortunately, long term use of L-dopa results in dyskinesias (involuntary movements). Current pharmacological therapies are symptomatic; none or retard dopaminergic neuron degeneration. Thus the development of animal models is essential for better understanding of the pathogenesis and progression of PD and discovering therapeutics to treat PD.[29,31]

2.2. DEFINITION

Parkinsonism is a clinical syndrome characterized by It is characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc).[29]

2.3. SIGNS AND SYMPTOMS

Parkinson's disease affects movements (motor symptoms). Typically other symptoms include disorders of mood, behavioural, thinking and sensation (non-motor symptoms).

2.3.1. Motor manifestations

Tremor: maximal when the limps are at rest, and decreased with voluntary movement.

Rigidity: stiffness or resistance to passive movement by limbs.

Bradykinesia: slowness and paucity of movement.

Postural instability: Failure of postural reflexes, which leads to impaired balance and falls.

Other motor symptoms include:

Gait freezing: motor block; freezing occurs as a sudden inability to step forward while walking. It is transient, last for seconds or minutes, and suddenly abates.

Dystonia: abnormal, sustained, painful twisting muscle contraction, often affecting the foot and ankle (mainly toe flexion and foot inversion).

Hypotphonia: soft speech; speech quality tents to be soft, hoarse, and monotonous.

Dysphagia: impaired ability to swallow; can lead to aspiration, pneumonia.

Masked faces (a mask like face, also called hypomania), with infrequent blinking.

Micrographia (small, cramped handwriting)

Impaired fine motor dexterity and motor coordination.[32,33,36]

2.3.2. Cognitive and psychiatric manifestitations

Dementia: slowing of thought and progressing to difficulties with abstract thought, memory and behavioural regulation.

Depression: about 47% of PD patients show evidence of depression.

Short term memory loss.

Hallucination, delusions, irritability, apathy and anxiety.[36]

2.4. DIFFERENTIAL DIAGOSIS OF PD [37]

Diagnosis	Historical features	Signs/symptoms	Radiographic finding
Idiopathic Parkinson's disease	Difficulty with tasks, rigidity, tremor	Tremor, rigidity, bradykinesia, loss of balance, micrographia	No specific CT or MRI findings
Drug induced parkinsonism	Previous use of antipsychotics, reserpine, metaclopramide	Tremor, rigidity, bradykinesia, often bilateral symptoms	Normal
Vascular parkinsonism	Stepwise progression; CVA or TIA, co morbid cardiovascular disease	Fixed deficits from previous events	Lesions in white matter with or without basal ganglia
Essential tremor	History in multiple family members, little evolution	Tremor often bilateral, mild rigidity; no extra pyramidal symptoms; no response to L-dopa	SPECT shows normal dopaminergic system
Progressive supranuclear palsy	Onset after 40 years of age; frequent falls	Vertical gaze paralysis, postural instability, resting tremor, dystonia, normal olfaction	MRI shows mesencephalon brain stem atrophy involving the superior colliculi

Table 1 : Differential diagnosis of PD

2.5. NEUROPATHOLOGY OF PD

Parkinsonism is a progressive motor disease that affects 1.5 million Americans. It is the second most common neurodegenerative disease after Alzheimer's and PD affects close to 5% of the population that are over 65 years old. The degeneration of dopaminergic neurons in the substantia nigra lead to PD. Due to abnormal protein folding and ER stress, a toxic protein named Lewy bodies are formed that are commonly observed in PD patients. This toxic protein, Lewy bodies, is made of different proteins such as α-synuclein, synphilin-1, and ubiquitin.

Lewy bodies have also been observed in other areas of the brain such as hind brain, spinal cord and enteric nervous system. Lewy bodies first appear in the periphery, subsequently it travels to brain stem and eventually in the cortex. Although there is some controversy as to whether all PD patients do have α-synucleinopathy, all α-synucleinopathies have one thing common and that is pathology found in discrete location and clinical manifestations may be due prionopathy that arose from different regions of the brain. Thus, a misfolded _-synuclein can trigger a downstream cascade of events that may lead to clinical symptoms.

There are other forms of PD that involve abnormally phosphorylated tau such as progressive supra nuclear palsy, cortico-basal degeneration, parkinsonism-dementia complex, and fronto-temporal dementia with Parkinsonism.[28,29]

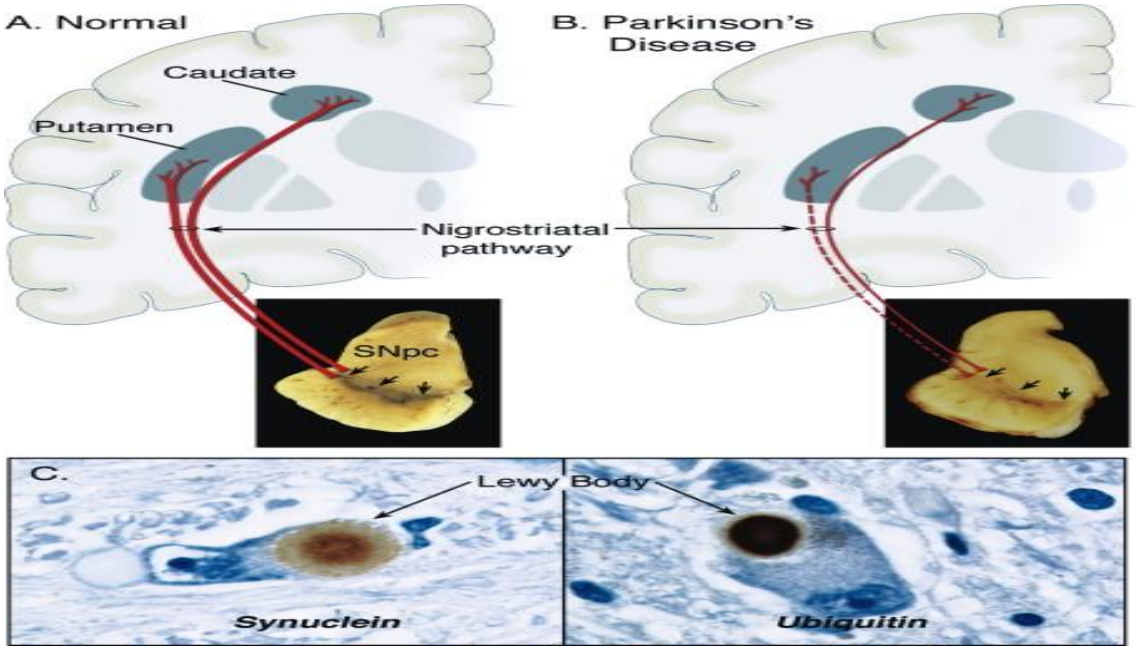


Figure 1: Neuropathology of PD

A. Normal nigrostriatal pathway (in red)- It is composed of dopaminergic neurons whose cell bodies are located in SNpc .These neurons project (thick solid red lines) to the basal ganglia and synapse in the striatum (i.e. putamen and caudate nucleus). The photograph demonstrates the normal pigmentation of the SNpc, produced by neuromelanin within the dopaminergic neurons.

B. Diseased nigrostriatal pathway (in red)- In PD, there is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a modest loss of those project to the caudate (thin red solid line). The photograph demonstrates depigmentation (i.e., loss of dark brown pigmentation neuromelanin) of SNpc due to the marked loss of dopaminergic neurons.

C. Immunohistochemical labelling of lewy bodies in SNpc dopaminergic neuron. Immunostaining with an antibody against α-synuclein reveals a LB (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Immunostaining with an antibody against ubiquitin yields more diffuse immunoreactivity within LB (right photograph).[29]

2.6. EPIDEMIOLOGY OF PD

PD is the most common neurodegenerative disorder of mid to late life in developed nations. During final decades of last century, in U.S. and Western Europe, 1 in 200 persons aged around 60-69 had PD. For people in their 70's this increased to 1 in 100 persons and for the people in their 80's in every 35 persons had PD. In developing countries like China in the 1980's only 1 in 1000 persons in their 60's was expected to have PD, but recent studies states that the prevalence is similar to that in U.S.

In among the five most populous nations, estimate of the number of individuals with PD over age the 50 was found to between 4.1 and 4.6 million in 2005 and 6.2 in 2015. This is expected to 8.2 million , by the year 2030.[38]

Population of elderly Indians has increased from 5.6% (51 million) in 1961 to 7.1% (71 million) in 2001. This increasing life expectancy of Indians, in the last decade, is likely to result in an age related disease like PD and Alzheimer disease. 355 million (61%) elderly people out of 580 million lives in developing countries, and of these 77 million (22% of total) lives in India. It was estimated that 33 million Indians do have neurological disorder. It was found that, the prevalence of PD was more in rural (41/105) than urban (14/105) population and was commoner among men. [39]

2.7. RISK FACTORS OF PD

2.7.1. Environmental factors

1-methyl-4-phenyl,2,3,6-tetra hydropyridine (MPTP)[30]

MPTP is a by-product of the chemical synthesis of narcotic meperidine analogue. It is highly lipophilic; easily crosses the BBB and converts to 1-methyl-4-phenyl-2,3-dihydropyridinum ion (MPTP+) via monoamine oxidase B within non dopaminergic cells and then to MPP+. MPP+ is transported to dopaminergic neurons by DAT and therefore exhibits selective toxicity to dopaminergic neurons. People intoxicated with MPTP develop a syndrome identical to PD.

Pesticides[31]

Human epidemiological studies have implicated residence in a rural environment and related exposure to herbicides and pesticides with an elevated risk of PD. Paraquat is a member of chemical class bipyridyl derivatives, has been used as herbicide. It is structurally similar to MPP+. Exposure to a combination of paraquat and maneb, which is also a pesticide, exacerbates dopaminergic degeneration in the rodent model and cause higher incidence of PD in humans. Rotenone, which is used as an insecticide is a potent inhibitor of complex I. it disrupts mitochondrial function and plays important roles in nigral cell degeneration in PD.

Caffeine, cigarette[40]

Cigarette smokers had 60% lower risk for the development of PD than people without a history of smoking. Several studies revealed that cigarette smoking inhibits monoamine oxidase activity and nicotine stimulates dopamine release. Thus it suppresses free radical generation and protects against dopaminergic cell death. Similarly coffee drinking could result in a 30% decreased risk of PD compared with non coffee drinkers. Caffeine had similar effects as A2A antagonist.

Disease process and dual hit theory[40]

Neuronal degeneration in PD may extend from peripheral systems, such as olfactory and autonomic systems to cortices. Dual hit hypothesis suggests that neurotropic pathogens may enter the nervous systems via both nasal and intestinal epithelium and this may promote α-synuclein aggregation.

2.7.2. Genetic factors

Synuclein

α-synuclein is a 140 aminoacid protein that is abundantly expressed throughout the brain, and especially in presynaptic nerve terminals. Two missense mutations [Ala53 ⟶ Thr (A53T) and Ala30 ⟶ Pro (A30P)] in α-synuclein cause dominantly inherited PD. In straiatal dopaminergic terminals, α-synuclein participates in the modulation of synaptic functions. α-synuclein is abundant in LBs. it's misfolding and formation of amyloid fibrils may be responsible for the neurotoxicity. Aggregation of α-synuclein is thought to be a key event in dopaminergic neuronal cell death in sporadic PD.[32]

Parkin

Parkin, a 465 amino acid protein, contains two RING finger domains separated by an in-between RING (IBR) finger domain at the c terminus and an ubiquitin-like homology domain at the N terminus. Parkin is an E3 ubiquitin ligase, a component of ubiquitin-proteasome system that identifies and targets misfolded proteins to the proteasome for degeneration. Heterozygote mutations in parkin may lead to dopaminergic dysfunction and later onset of PD. [40]

Ubiquitin C-Terminus Hydrolase-L1

A dominant mutation (193M) in UCH-L1 was identified in one family with inherited PD, where as polymorphism (S18Y) of UCH-L1 appears to be protective for the development of PD. UCH-L1 ligase activity is decreased by the pathogenic 193M mutation and increased by the protective S18Y polymorphism.[40]

DJ-1

DJ-1 appears to protect cells against mitochondrial complex-1 inhibitors and oxidative stress induced by hydrogen peroxide. This effect is abrogated by DJ-1 mutations or by DJ-1 knockdown using SiRNA. DJ-1 can act as an antioxidant.[29]

PINK 1 gene

Missense and truncating mutations in PINK 1 gene found to cause autosomal recessive PD. PINK 1 protein consists of a kinase domain and a mitochondrial targeting motif. Its neuroprotective property os lost by G309D mutation which is seen in certain families.[40]

LRRK2

LRRK2 mutations were identified as the causative gene for PARK8- linked familial PD. R1628P and G2385R are polymorphic mutations and have been demonstrated as risk factors for sporadic PD in Asian populations. An LRRK2 mutation causes neuronal loss in substantia nigra accompanied by LBs.[41]

2.8. PATHOGENESIS OF PD

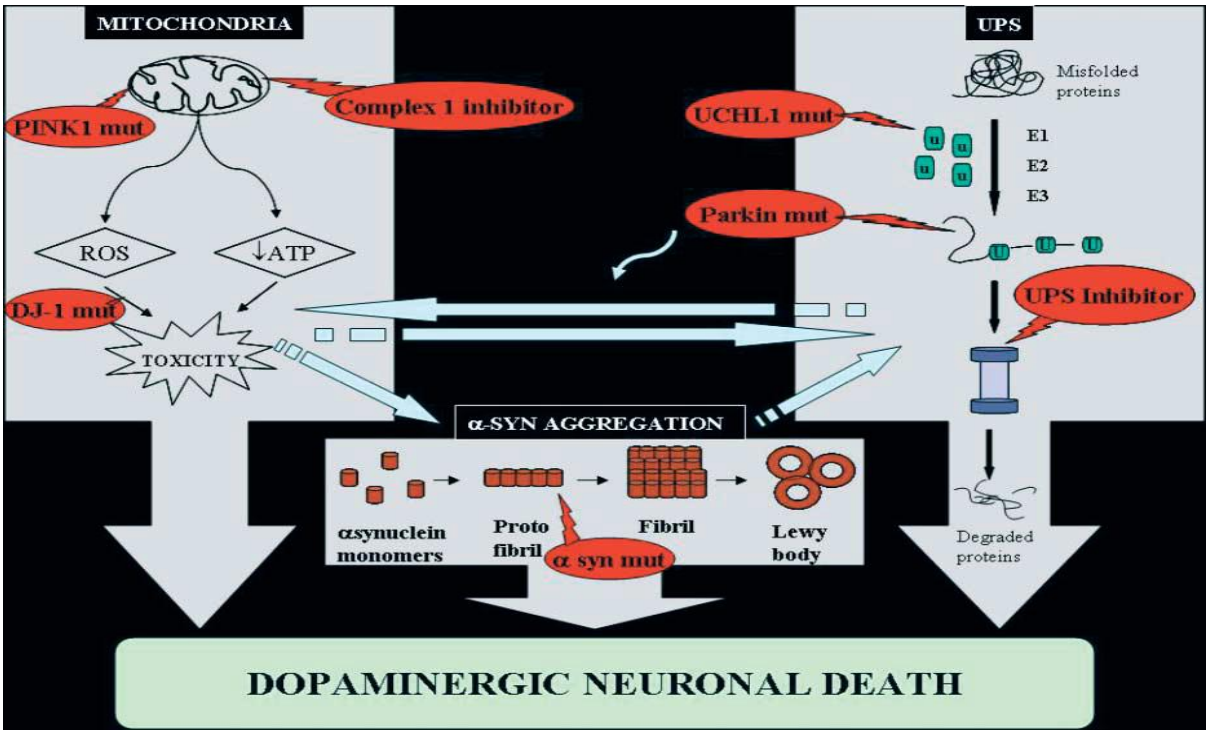


Figure 2: Mechanism of neurodegeneration

2.8.1. Misfolding and aggregation of proteins

Accumulation of misfolded proteins is a key event in PD neurodegeneration. Protein aggregates could directly cause damage or by deforming the cell or by interfering with intracellular trafficking in neurons. Pathogenic mutations may directly induce abnormal protein conformations (α-sunuclein) or damage the ability of the cellular machinery to detect and degrade misfolded proteins (Parkin, UCH-L1). Oxidative damage, linked to mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations.[29,32]

2.8.2. Mitochondrial dysfunction and oxidative stress

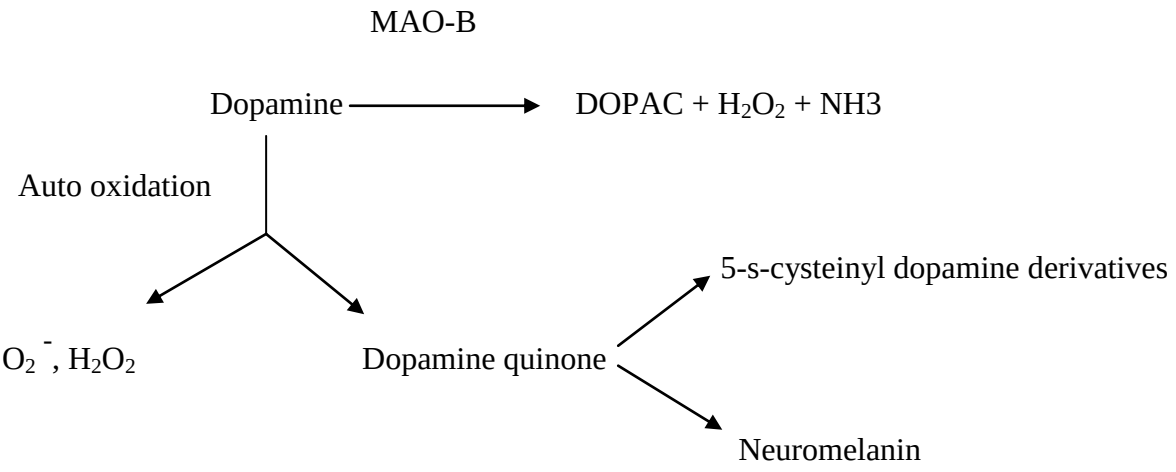
Oxidative phosphorylation defect play a role in the pathogenesis of PD. This is supported by finding that MPTP blocks the mitochondrial electron transport chain by inhibiting complex I. In vitro studies indicate that complex I defect may subject cells to oxidative stress and energy failure. Inhibition of complex I increases the production of the ROS superoxide.

Increased reactive oxygen species (ROS) production from dopamine metabolism or dysfunctional mitochondria may induce cellular damage and death through modification of nucleic acids, proteins and lipids. Age related depletion of glutathione levels precipitates the accumulation of oxidizing free radicals such as hydrogen peroxide, hydroxyl ions and superoxide in the brain.

Dopamine produced by nigral neuron leads to both mitochondrial dysfunction and impaired proteolysis. Metabolism of DA produces hydrogen peroxide and superoxide radicals and auto-oxidation of DA produces DA-quinone, a molecule that damage protein or further converted to neuromelanin. The modification of bio molecule by hydroxyl radicals or dopamine quinine exerts toxic effect on dopaminergic neurons.[41,42]

2.8.3. Programmed cell death

Activation of programmed cell death machinery believed to be a factor that triggers the death of dopaminergic neurons in PD. In PD, SNpc dopaminergic neurons with increased expression of anti-PCD protein Bcl-xl and activated PCD effector protease caspase 3 have been found. Other molecular markers of PCD altered included activation of caspase-8 and caspase-9. [32]



2.9. ANIMAL MODELS OF PD

Systemic administration

MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
PARAQUAT
ROTENONE

Local administration

6-HYDROXY DOPAMINE
LIPOPOLYSACCHARIDE

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

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), is a potent neurotoxin & highly lipophilic. After systemic administration it rapidly crosses the blood-brain barrier, enters astrocytes and is metabolized to its active metabolite MPP+ by monoamine oxidase-B (MAO-B). MPP+ is able to inhibit complex 1 of the mitochondrial electron transport chain, resulting in the formation of ROS & leading to reduced ATP production.

Neuroprotection suggests preventing or slowing disease progression. Nevertheless, despite advances toward this goal, all current treatments are symptomatic; none halt or retard dopaminergic neuron degeneration.[33]

2.9.2. ROTENONE

PD can be induced with mitochondrial complex I inhibitors such as the environmental toxins rotenone. Rotenone, a commonly used natural pesticide prepared from the roots of tropical plants, such as Derris elliptica, can freely cross cell and mitochondrial membranes. *In vitro*, rotenone has been shown to promote the accumulation and aggregation of alpha-synuclein and ubiquitin, cause oxidative damage, and endoplasmic reticulum stress, and lead to cell death. *In vivo*, chronic exposure of rats to rotenone induces PD-like symptoms, including dopaminergic neurodegeneration and the occurrence of cytoplasmic inclusions similar to Lewy bodies. Re- cent studies show that chronic exposure of Drosophila to rotenone recapitulates key features of Parkinsonism, including selective loss of dopaminergic neurons and locomotor deficits. Although there are also study showing contradictory results, most of the evidences are consistent and suggesting that rotenone exposure contributes to PD-like symptom and that rotenone-based Parkinson's disease models can be used to test potential compounds for PD intervention.[54]

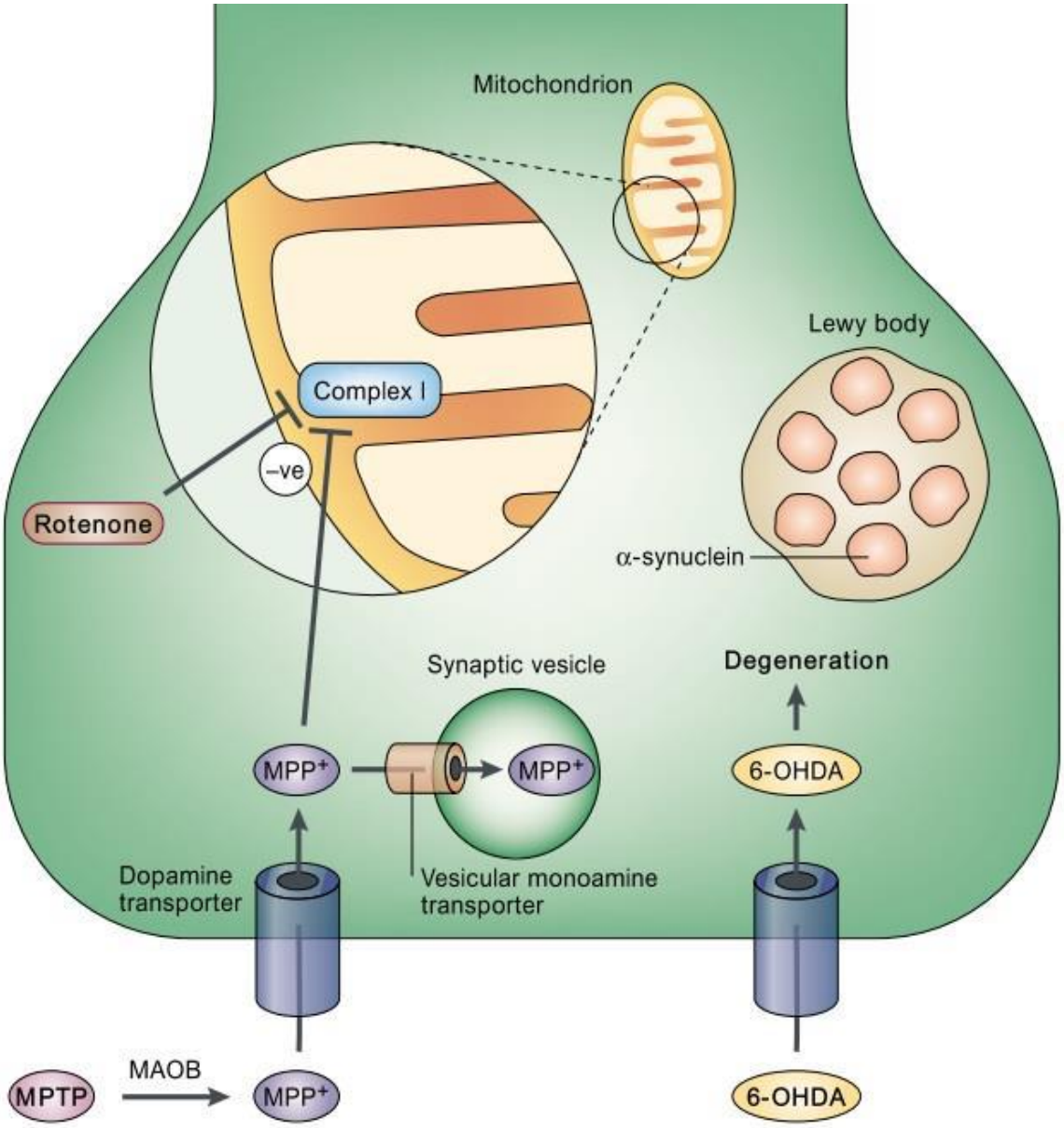


Figure 3: Effects of toxins used in model of Parkinson's disease on dopamine cells

2.10. DRUG CLASSES FOR PARKINSONISM[43]

I. Drugs affecting brain dopaminergic system

- Precursors of Dopamine: Levodopa
- Inhibitors of Dopamine metabolism:
  - ❖ COMT inhibitors: Tolcapone, entacapone
  - ❖ MAO-B inhibitors: Selegiline,rasagiline
- Dopamine releasers: Amantadine
- Dopamine receptor agonists:
  - ❖ Ergot derivatives: Bromocriptine, Pramipexole, Ropinirole
  - ❖ Non-Ergor derivatives: Lysuride

II. Drugs that affect brain cholinergic system

- ❖ Central anti-cholinergics: Procyclidine, benzhexol
- ❖ Antihistaminics: Promethazine, orphenadrine

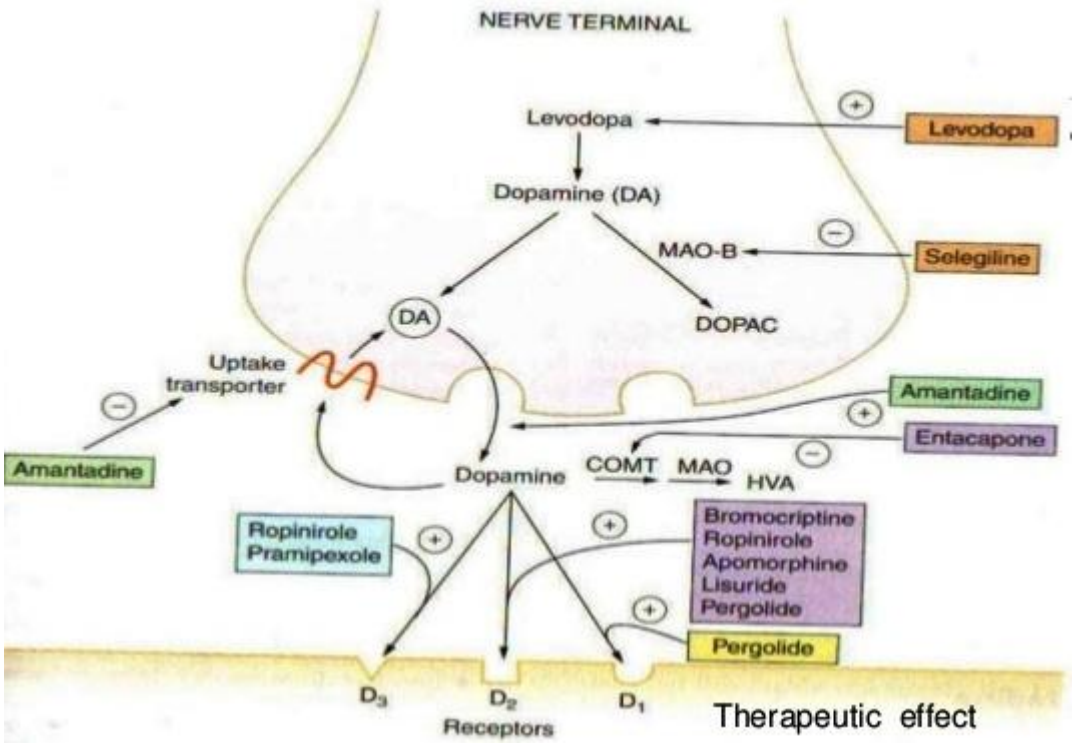
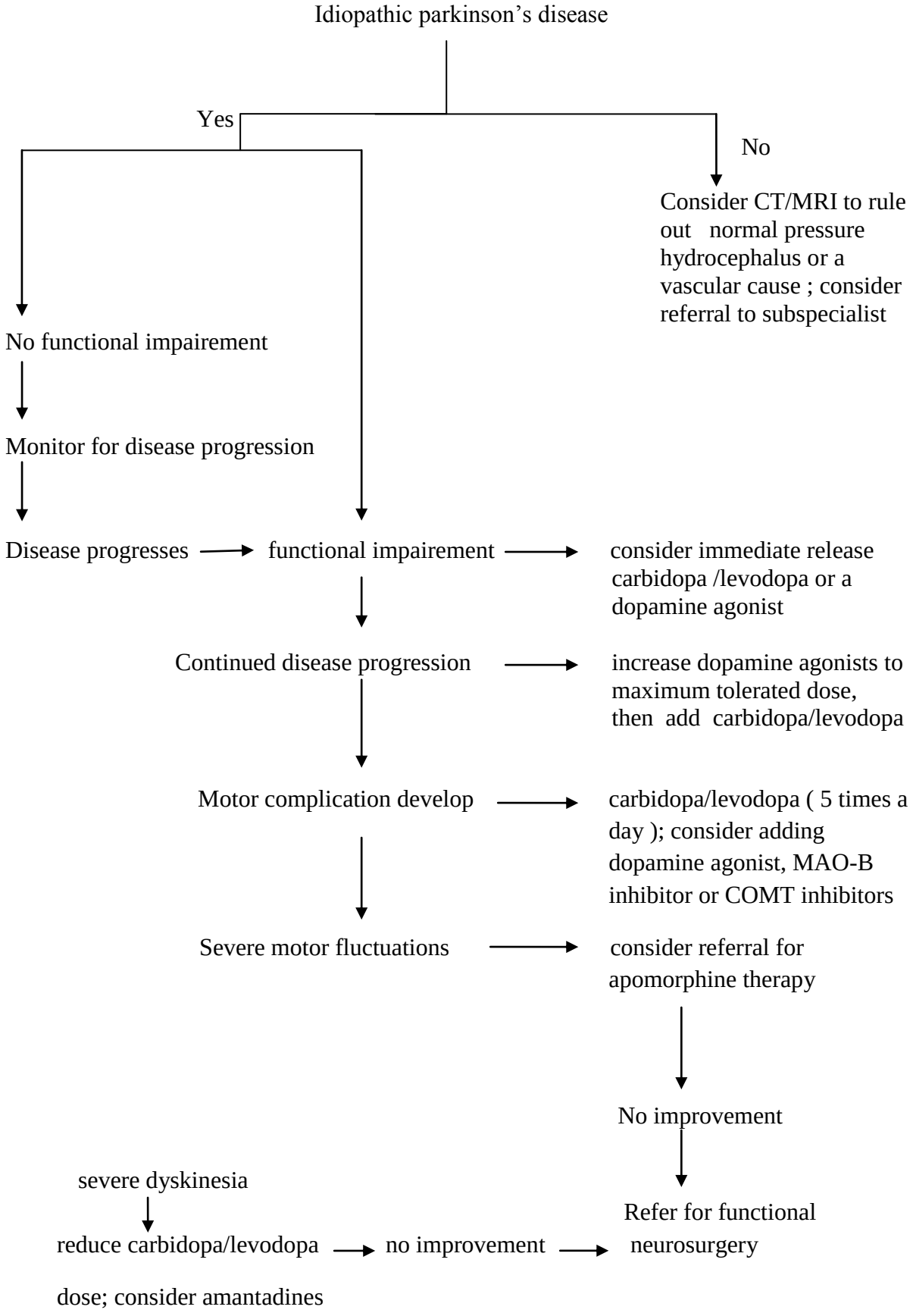


Figure 4: Site of action of current therapies

2.11. MANAGEMENT OF PD[37]



2.11.1. Limitation of current therapies[43]

Levodopa

The gold standard of PD therapy; acts as aprecursor of dopamine. When levodopa is administered orally it is rapidly decarboxylated; thus high dose is required for desired effect which induces nausea and vomiting in patients. Longterm treatment of levodopa results in adverse motor effects which limits its use including on-off phenomenon, wearing off, dose failure, akinesia and dyskinesias.

Selegiline

This MAO-B inhibitor prevents the in vivo metabolism of dopamine. Its main use is as adjuntive therapy with levodopa which may lead to potentiated side effects. Studies suggest that selegiline may retard disease progression and delays the need for levodopa. However, therapeutic effect is mild when used alone.

Amantadine

Amantadine, an antiviral agent was found by chance to be effective in PD. It is effective in reducing diskinesias. Its CNS effects include restlessness, depression, confusion and hallucinations.

Anticholinergics

Trihexyphenidyl or benztropine are specifically effective against tremor. Side effects such as confusion, drowsiness, agitation and hallucination are common. Drug withdrawal leads to precipitation of acute parkinsonian symptoms.

Dopamine receptor agonists

They may be used alone to delay the need for levodopa or used with levodopa to increase their effectiveness. The ergot derivatives causes psychiatric disturbances and cardiovascular problems that can progress to myocardial infractions. Even at lower doses patients experience orthostatic hypotension, constipation, dyskinesias, confusion and insomnia.

COMT inhibitors

COMT inhibitors are used mainly in combination with levodopa. The incidence of sleep disturbances, orthostatic hypotension, dyskinesias, confusion and insomnia. Tolcapone produce heparotoxicity and has been restricted in many countries.

2.11.2. Newly researched neuroprotective agents

Nicotine

Nicotine, principle alkaloid in tobacco evokes the release of dopamine from the striatum; thus it protects nigrostriatal neurons from degeneration. It acts by the activation of presynaptic nicotinic α4, β2 and α7 receptors located on the dopaminergic nerve terminals. It also acts as free radical scavenger. [44]

Anti-inflammatory agents

The enzyme cyclo-oxygenase (COX) as well as inflammatory mediators like nitric oxide were reported to increase in PD. Non-selective COX-inhibitors, aspirin and the COX-2 inhibitors meloxicam were reported to confer neuroprotection in MPTP induced dopamine depletion in mice. Salicylic acid acts as free radical scavengers in the brain, but its neuroprotective ability is independent of prostaglandin mediation.[45]

Melatonin

Melatonin, a serotonin derivative is a hormone synthesized naturally by neurons in the pineal gland. It has antioxidant properties and act as a free radical scavenger. It causes dose-dependent reduction in the production of dopaminergic neurodegenerating hydroxyl free radicals.[46]

Monoamine oxidase-B (MAO-B) inhibitors

Rasagiline, a noval MAO-B inhibitors potentially reduces the progression of PD. It is effective in alleviating symptoms of PD. Youim et al., (2005) devolped a compound, Ladostigil which has both ChE and MAO-B inhibitory activity; a potential treatment for PD patients with dementia.[47]

Selenium

Selenium, an essential trace metal present in the body has been found to be effective in delaying neurodegeneration due to its role in the functioning of the antioxidant enzyme glutathione peroxidise (GPx).[48]

Iron-chelators

The use of iron chelators including desferoxamine and the newer agent V-28; and combination with MAO-B inhibitors, rasagiline yielded reduction in the degeneration and formation of protein aggregates. They acts by interfering α-synuclein iron reactivity or by reducing iron content in the substantia nigra.[49]

Vitamin A, C and E

Levels of 4-hydroxy-2,3-noneal (HNE), an aldehyde generated during lipid peroxidation and 8-hydroxyguanosine, a nucleoside oxidation product are increased in substantia nigra of PD patients. Vitamins A, C and E are proven antioxidants which prevents lipid peroxidation. Treatment with high doses of Vitamin A and C delayed the use of levodopa or DA agonists by 2.5 years.[50]

2.11.3. Non-pharmacological treatment

Gene therapy

Genes those encoding tyrosine hydroxylase, guanosine triphosphate cyclohydrolase I and aromatic L-amino acid decarboxylase allow for an increase in the production of dopamine. The differentiation of these neuron is mediated by agents such as transforming growth factor beta (TGF-β) and bone morphogenic factors (BMPs). Thus far, glial cell line-derived neurotrophic factors (GDNF) have proven to be the most promising option to restore dopaminergic activity in substantia nigra. Proposed systems have ranged from adenoviral and lentiviral nigrostriatal implants to liposomes.[50]

Surgical methods

Prior to the commercial availability of levodopa, treatment for PD emphasized surgical intervention. Surgery is considered for people with intolerable adverse side effects from medication, and those who have significant cognitive capacity.

Pallidotomy- destruction of a small portion of the brain that is overactive, by use of an electric probe. This primarily reduces dyskinesia; minimal effects on bradykinesia and rigidity.

Thalamotomy- involves the removal of the thalamus in the brain. This procedure is rarely performed and considered as safe and effective to treat tremor.

Thalamic stimulation- involves the insertion of an electrode wire into the thalamus, the other end connected to a pulse generator placed under the skin in the thorax; effective in the management of tremor in PD.

Deep brain stimulation- An alternative procedure used to destroy small region in the brain. A thin electrode implanted into the brain prevents transmission of impulse for involuntary movements.[51]

2.11.4. Herbal remedies

Herbal medicines have been main source of primary health care in all over the world. About 80% of the world populations are still dependent on traditional medicines. The lack of effective and widely applicable pharmacological treatments in the modern therapy for neurodegenerative disorders may explain a growing interest in the traditional medicines. In Indian medical system Ayurveda, "Kambaveta" is the term used to explain Parkinson's syndrome. In recent years, increasing interest has been devoted to the treatment of PD by herbal medicines.[53,54]

***Mucuna pruiens*; (Family: leguminosae)**

Mucuna pruiens commonly known as velvet bean or the cowhage is used in spasms associated with Parkinsonism. Its seeds are well known to contain levodopa and possess androgenic, analgesic, anti-inflammatory, antiparkinson's and antispasmodic activities MP extract shows twice the antiparkinson's activity compared with synthetic levodopa in animal models.[53]

***Acanthopanax senticocus* Harms; (Family: Alariaceae)**

Ethanol extract of *Acanthopanax senticocus* stem bark at dose of 250mg/kg shows prophylactic effect on behavioural dysfunction of parkinsonism such as bradykinesia, catalepsy, depression by significant increase in the dopamine level in the striatum in MPTP models.[54]

***Ginko biloba*; (Family: Ginkgoaceae)**

The neuroprotective effects of a standardised extract of *Ginko biloba* were investigated on 6-OHDA induced neurotoxicity in rat. The pre-treatment EGb for 3 weeks cause significant restoration of striatal dopamine and its metabolites, it is a potent inhibitors of MAO-B. The loco motor deflects were restored and causes increase in the content of GSH.[52]

***Withani somnifera*; (Family: Solanaceae)**

Withani somnifera have significant role in prevention of various CNS disorders, particularly in epilepsy, stress and neurodegenerative disease such as Parkinson's and Alzeimer's disease. The root extract at dose of 100mg/kg shows significant improvement in motor neuron function, catecholamines and antioxidant level; prevent lipid peroxidation.[52]

***Hypericum perforatum*; (Family: Hypericaceae)**

Pre treatment with standardised extract *Hypericum perforatum* for 45 days in rotenone exposed rats, exerts an antioxidant action which was related with decrease of MnSOD activity, increase SOD and CAT activity and protect the cells from damaging effect of H_2O_2; shows neuroprotective activity.[53]

2.12. *Barleria Prionitis* linn

Piush Sharma, et al; (2013) investigated and reported the phytochemical and ethanomedical values of *Barleria prionitis L*.(Acanthaceae) which have been established against various disease models using modern scientific methodologies and tools. They concluded that *Barleria prionitis* is abundant in terms of presence of phytoconstituents and their active secondary metabolite. The reported pharmacological activity include anti-fertility activity, anti-oxidant activity, anti-inflammatory, hepatoprotective, anti-diabetic activity, anti-dental decay activity and anti-microbial activity.[54]

Nidhi et al., (2013) evaluated the antibacterial activity of rhizome of *Barleria prionitis* in methanol extract. The antibacterial potential was measured by agar disc plate method. The methanol extract showed antibacterial activity against two Gram's positive and two Gram's negative bacteria. By using Gas chromatograpy with mass spectrophotometric detector the active phytocomponents of *Barleria prionitis* were revealed and 27 constituents identified. All the results supported that the extract can be used to prevention of bacterial infection.[55]

Manider Karan *et al.*, (2013) assessed topical anti-inflammatory activity of *Barleria prionitis* and *Barleria Cristata* against croton oil induced ear oedema in female rats. Different extract using methanol, hexane, chloroform, ethyl acetate, butanol and aqueous extract of each Barleria species were evaluated at a dose of 200-400 mg/ml prepared in croton oil solution. From the above study they concluded that chloroform extract of *Barleria prionitis* showed best topical activity with 88.31% inhibition of ear oedema..[56]

Kuldeep Singh *et al.*, (2013) evaluated the anti-inflammatory effect of methanol extract of *Barleria prionitis* on carragennan induced rat paw oedema. They concluded that methanolic extract of *Barleria prionitis* for the dose of 500 mg/kg p.o shown anti-inflammatory activity in the early stage as well as in the late stage of the study. The inhibition of Carragennan induced paw oedema by crude extract is due to inhibitory activity of lipoxygenase enzymes.[57]

Manjusha *et al.*, (2013) evaluated the gastro protective activity of methanolic extract of *Barleria prionitis* on ethanol and indomethacin induced ulcer models in rats. Parameters like volume of gastric juice, pH, free acidity, total acidity, AST and ALT were determined. The reduction in ulcer index in *Barleria prionitis* treated animal was found to be statistically significant when compared with control groups in both the model. They concluded that the methanolic extract of *Barleria prionitis* possess antiulcer activity, which supports the traditional use of this plant in treating gastric ulcer.[58]

Swathi Paul. *et al.*, (2012) reported the potent antibacterial activity of different leaf extracts of *Barleria proinitis* L. They studied the antibacterial effect and the minimum inhibitory concentration (MIC) using different extract (petroleum ether, chloroform, ethanol (70%)) and column fraction of *Barleria prionitis* leaf. S. Typhi, V. Cholera, M. Lute, L. sporogenus and Citrobacter were inhibited at 5mg/ml level. B. subtils inhibited at 3.33 mg/ml level. B. cereus and Providencia were inhibited at 50 mg/ml.[59]

Reema Dheer *et al*., (2011) studied the antibiotic activity of alcoholic extract of leaf and root of *Barleria prionitis* in normal and alloxan induced diabetic rats. The alcoholic extracts of leaves and alcoholic extracts of roots of *Barleria prionitis* were given at a dose of 200 mg/kg for 14 days. They concluded from the study that animals treated with the alcoholic extract of leaves of *Barleria prionitis* significantly decreased the blood glucose level and glycosylated haemoglobin and an increase in serum insulin level and liver glycogen, where as decrease in weight was arrested. The alcoholic root extract showed a non significant antidiabetic activity in experimental animals.[60]

Chavan Chetan *et al*., (2011) investigated the antioxidant activity of ethanolic and aqueous extract of whole plant of *Barleria prionitis*. The study indicated that the ethanol and aqueous extract of *Barleria prionitis* possesses significant antioxidant activity. The order of antioxidant potency of the whole plant extract is ethanol > aqueous. The phenolic content of was determined using Folinciocalteau reagent. They concluded that the presence of high levels of phenolic compounds in the ethanolic extract contributed to the observed antioxidant activity.[61]

R.M.Santiago *et al*., (2010) reported the depressive like behaviour and neurotransmitter alterations in PD models induced by intranigral injection of MPTP, 6-OHDA and rotenone. The data indicate that MPTP, 6-OHDA and rotenone were able to produce anhedonia and behavioural despair. These altered behavioural responses were accompanied by reductions of striatal DA, homovanillic acid (HVA) and DOPAC restricted to the 6-OHDA group. Additionally, decreases on the hippocampal serotonin (5-HT) content were detected for the MPTP, 6-OHDA and rotenone groups.[62]

Ramya kubar B *et al*., (2011) evaluated the neuroprotective effect of various extracts of Prosopis chilenis (PC) seeds in MPTP mouse models. PC seed extract was administered at different doses in different groups once a day for seven days and the first dose was given 30 min prior to first MPTP injection (20mg/kg/i.p; 4 injections at 2h intervals).The alcoholic extract at given doses [100,200 and 300mg/kg (p.o)] significantly dose dependently increased the spontaneous motor activity, grip strength and alertness. Alcohol, ethyl acetate and aqueous extract of PC significantly improved the brain dopamine, nor epinephrine, epinephrine and 5-HT at a dose of 200 and 300mg/kg. These results indicate that PC extracts had neuroprotective effect on MPTP induced PD.[63]

2.13. PLANT PROFILE[64-67]

BARLERIA PRIONITIS

Plant name : *Barleria prionitis* also known as porcupine flower.

Family : Acanthaceae

Vernacular Names

Hindi : Vajradanti

Sanskrit : Karunta

English : Common Yellow Nail Dye Plant

Bengali : Kantajati

Tamil : Shemuli

Malayalam : Manjakanakambaram

Morphological characters:

Barleria prionitis is a branched annual shrub of about 1-3 feet height with flower and is a spiny invader. The spines in leaf axils are 5-20mm long. Flowers are yellow or whitish in color, 3-4cm long and broad, tubular, sessile in leaf axils. The white flower variety is bitter in taste. The stem is generally single, but may have multiple stems or branches near the ground. The stem branches are stiff, round, light gray colored and globrous. The roots are tap like and with lateral system. The leaves are elliptic to oblong, up to 3-10 cm long and 15.4 cm broad. The seeds of Barleria prionitis are compressed, about 8mm length and 5mm width. The seeds are flattened and covered with matted hairs. The fruits are ovoid and capsule shaped.

Geographic distribution

The *Barleria prionitis* is native to tropical areas of east Africa and Asia, also found throughout tropical Asia such as India and Srilanka and in south Africa also.

Ecology

Porcupine flower or *Barleria prionitis* grows in a wide variety of well drained soils derived from igneous, metamorphic, and sedimentary rocks. It grows in areas receiving from about 750 to 900 mm of mean annual precipitation. Porcupine flower is found throughout the hotter parts of India and defoliates annually during the dry season. The species is moderately intolerant of shade, growing in both full sunlight and under light forest canopies.

Growth and Management

The plant *Barleria prionitis* flower from September to December and fruit from January to April. Forty-four percent of the seeds sown on commercial potting mix germinated between 13 and 77 days following the sowing. Matured plants add up to 0.5 m / year to their height and nursery seeding reach 0.6 m in 6 months. The shrub lives about 4 years.

Chemical constituents

Barleria prionitis consist of acabarlerin, barlerin, β- sitosterol, flavanol, glycoside, iridoids and scutellarein-7-neohesperidoside.

Aerial parts of *Barleria prionitis* consist of phenyl ethanol glycoside ,barlerinoside along with six iridoid glycosides, shanzhiside methyl ester, 6-o-trans-p-coumaroyl-8-o-acetyl shanzhiside methyl ester, barlerin, acetyl barlerin, 7-methoxy diderroside, luplinoside. Also prinoside A, prinoside B, prinoside C.

Traditional uses

Traditionally the plant is used for asthma, whooping cough and the leaves and roots are used against toothache, rheumatism, cataract, leucoderma, scabies, liver ailments, piles treatment, diuretic, ulcers and also in cut, wounds, malaria, fever and irritation control.

Pharmacological activity

Anti-fertility activity, anti-oxidant activity, anti-inflammatory activity, hepatoprotective activity, anti-microbial activity, anthelmintic, anti dental decay activity, anti-diabetic activity, anti-diarrhoeal activity, diuretic activity, besides these it has got AChE inhibitory activity, anxiolytics.

Figure 5: ***Barleria Prionitis linn***

3. METHODOLOGY

3.1 COLLECTION AND AUTHENTICATION OF PLANT

The whole plant of *Barleria prionitis*were collected from the surrounding areas of Erode district, Tamilnadu, India during the month of Decemberand authenticated by Botanical survey of India (BSI) southern circle, Coimbatore, Tamilnadu. The authentication certificate number is No.BSI/SRC/5/23/2016-14/TECH/2108. Soon after collection the aerial parts were cleaned, driened in shade and crushed to a coarse powder, stored in an air tight plastic container, until further use.

3.2 EXTRACTION OF PLANT MATERIAL

Coarsely powdered whole plant of *Barleria prionitis* were extracted with water for 48 hours at room temperature. After extraction the extracts were evaporated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4°C for the further analysis.

3.3. QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AEBP [68-71]

preparation of sample

A small quantity of extract was dissolved in 5ml of distilled water and then filtered. The filtrate was tested to detect the presence of different phytochemical constituents in the sample.

3.3.1. DETECTION OF CARBOHYDRATE:

Small quantity of extract was dissolved in 4ml of distilled water and filtered. The filtrate was collected and subjected for the following tests.

a. Molisch's test:

1 ml of filtrate was treated with 2-3drops of 1% alcoholic α-napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown to violet ring, indicate the presence of carbohydrate.

b. Fehling's test:

To the Fehling solution A and B extract was added and boiled. The formation of brick red precipitate indicates the presence of reducing sugar.

c. Benedict's test:

1 ml of extract was added to 5ml of Benedict's reagent, was added and boiled for 2 mins and cool. Formation of a red precipitate shows the presence of sugars.

3.3.2. TEST FOR GLYCOSIDES:

a.Legal's test:

The filtrate was hydrolyzed with dilute hydrochloric acid and heated on water bath. Then added 1ml of pyridine and few drops of sodium nitroprusside solution, made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

b.Baljet test:

1ml of extract was added to 1ml of sodium picrate solution and the yellow to orange colour shows the presence of glycosides.

c.Keller killiani test:

To 2ml of extract, add glacial acetic acid, trace quantity of ferric chloride and add 2 to 3 drops of concentrated sulphuric acid. reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.

3.3.3. DETECTION OF ALKALOIDS:

Small quantity of extract was treated with few drops of dilute hydrochloric acid and filtered it. The filtrate was collected and subjected for tests with following reagents.

a. Mayer's reagent:

To the filtrate potassium mercuric iodide was added. The formation of cream colour precipitate, it shows the presence of alkaloids.

b. Dragendroff's reagent:

To the filtrate potassium bismuth iodide was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

c. Wagner's reagent:

To the filtrate iodine in potassium iodide solution was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

d. Hager's reagent:

To the filtrate saturated aqueous solution of picric acid was added. Formation of yellow precipitate indicates the presence of alkaloids.

3.3.4. DETECTION OF PHYTOSTEROL AND STEROIDS:

Small quantity of extract was dissolved in 5ml of chloroform and then subjected to the following tests.

a. Salkowski test:

To the above solution 1 ml chloroform and few drops of concentrated sulphuric acid was added. The test tube was shaken for few minutes. The development of red colour in chloroform layer indicates the presence of steroids.

b. Liebermann- Burchard reaction:

To the above solution 1 ml of chloroform and few drops of concentrated sulphuric acid and 1-2 ml of acetic anhydride were added. Development of red colour first, then blue and finally green colour, indicates the presence of steroids.

3.3.5. DETECTION OF PROTEINS AND AMINOACIDS

Small quantity of the extract was dissolved in few ml of water and filtered. The collected filtrate was used for following tests.

a. Biuret test:

Filtrate was treated with 5% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

b. Ninhydrin test:

To the filtrate Ninhydrin reagent was added. Development of violet or purple colour indicates the presence of amino acids.

3.3.6. DETECTION OF TANNINS:

a. Lead acetate test:

To 5 ml of aqueous extract was treated with 1 ml of 10% lead acetate solution. Yellow colour precipitation, indicates the presence of tannins.

b.Vanillin hydrochloride test:

1ml of extract was added with vanillin hydrochloride. Formation of purplish red colour indicates the presence of tannins.

3.3.7. DETECTION OF FLAVONOIDS

a. Zinc hydrochloride reduction test:

Treat extract with mixture of zinc dust and concentrated hydrochloric acid. Formation of red colour indicates the presence of flavonoids.

b. Alkaline reagent test:

To the extract, add few drops of sodium hydroxide solution. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

3.3.8. DETECTION OF SAPONINS

a. Foam test:

The extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

b. Froth test:

To 5 ml of test sample add few drops of sodium bicarbonate. Shake the mixture vigorously and keep it for 3 minutes. A honey comb like froth formation indicates the presence of saponins.

3.3.9. DETECTION OF TRITERPENOIDS

a. Libermann-Burchard test:

To the extract add few drops of acetic anhydride, followed by few drops of concentrated sulphuric acid. A brown ring forms at the junction of two layers and the upper layer turn green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

b.Salkowski Test:

5ml of extract was mixed in 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. Formation of reddish brown coloration of the interface indicates the presence of triterpenoids.

3.3.10. TEST FOR PHENOLS:

a.Ferric chloride test:

To 1 ml of extract, add 2 ml of distilled water followed by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

b. Lead acetate test:

Dilute 1 ml of extract with 5 ml of distilled water and to this add few drops of 1% lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenols.

3.4. EVALUATION OF ANTI-PARKINSON STUDY OF AQUEOUS EXTRACT OF *Barleria prionitis linn*

Table 2: Selection of animal for MPTP induced Parkinson evaluation

Species	Swiss albino mice
Age	3 month
Body weight	25-30 g
Gender	Male
No: of animals	30

Male Swiss albino mice 3 month of age, and 25-30 g body weight were offered by KMCH College of Pharmacy, Coimbatore. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee).

3.4.1. EXPERIMENTAL DESIGN FOR MPTP INDUCED PARKINSON

Group	Number of animals	Group Specifications
Group I	6	Vehicle control (normal saline i.p)
Group II	6	Only MPTP (25 mg/kg, i.p)
Group III	6	MPTP + Standard [Levodopa 12 mg/kg + Benzerazide 3mg/kg i.p]
Group IV	6	MPTP + AEBP (200 mg/kg, p.o)
Group V	6	MPTP + AEBP (400 mg/kg, p.o)

Table 3 : Experimental design for MPTP induced Parkinson

Table 4 : Selection of animal for ROTENONE induced Parkinson evaluation

Species	Sprague-Dawley
Age	50 days
Body weight	80-100
Gender	Male
No: of animals	30

Male Sprague- Dawley rats 50 days of age, and 80-100 g body weight were offered by KMCH College of Pharmacy, Coimbatore. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee).

3.4.2. EXPERIMENTAL DESIGN FOR ROTENONE INDUCED PARKINSON

Group	Number of animals	Group Specifications
Group I	6	Vehicle control (normal saline i.p)
Group II	6	Only ROTENONE (3 mg/kg, i.p)
Group III	6	ROTENONE + Standard [Levodopa 12 mg/kg + Benzerazide 3mg/kg i.p]
Group IV	6	ROTENONE + AEBP (200 mg/kg, p.o)
Group V	6	ROTENONE + AEBP (400 mg/kg, p.o)

Table 4 : Experimental design for Rotenone induced Parkinson

3.4.3 Induction of Parkinson[72,73]

Preparation and induction of MPTP solution

The MPTP was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (37^0C) to prevent its decomposition. The MPTP solution was freshly prepared at 25 mg/kg. The MPTP was dissolved in 0.9% sodium chloride solution and injected i.p at the dose of 25 mg/kg body weight, 7 days. MPTP solution is stable only for a period of 24 hours at 4^0C.

Preparation and induction of ROTENONE solution

The Rotenone was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (-20^0C) to prevent its decomposition. The Rotenone solution was freshly prepared at 3 mg/kg. The Rotenone was dissolved in DMSO and adjust to pH 7.4 with potassium hydroxide. Rotenone injected i.p at the dose of 3 mg/kg body weight, 7 days. The solution should be used immediately after preparation. Rotenone solution is stable only for a period of 24 hours at 25^0C.

Preparation of Levodopa and Benzerazide

12mg/kg of levodopa and 3 mg/kg of Benzerazide was dissolved in distilled water. Levodopa and benzerazide was freshly prepared daily and given via i.p to the standard group.

Preparation of sample

200 mg/kg and 400 mg/kg were dissolved in distilled water and it was prepared freshly and given via oral route to group IV& V respectively for 7 days.

3.5. EVALUATION PARAMETERS

3.5.1. MOTOR CO-ORDINATION TEST (ROTA ROD TEST)

Principle

The rota rod performance test is carried out on a rotating rod that provides forced motor activity in animals. The animals were placed on a rotating rod which is placed horizontally, suspended above a cage floor, which is high enough to induce avoidance of fall. Animals naturally try to stay on the rotating rod avoid falling to the ground. The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning.[74]

Procedure

Motor Co-ordination test was conducted using rota rod apparatus. Animal was placed individually on the rotating rod and trained for 3 min trail at 25 rpm on the day before the first day of testing. A cut off time of 180s was fixed and each animal performed 3 separate trials at 5 min interval. After each trial, 5 min rest period was given to alleviate stress and fatigue. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted.[75]

3.5.2. LOCOMOTOR ACTIVITY

Principle

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square area in which the animal moves.[76]

Procedure

The spontaneous locomotor activity of each animal was recorded individually, using Actophotometer. The apparatus was placed in a sound attenuated and ventilated room during the testing period. All the animals were placed individually in the activity cage for 3 min to

habituate them before starting actual locomotor activity task for the next 3 min. the basal activity score was noted. The units of the activity counts were arbitrary and based on the beam breaks by movement of the animal. Counts/3 min is used as an index of locomotor activity.[77]

3.5.3. FORCED SWIMMING TEST

Principle

Forced swimming test Is a behavioural despair test. This test is most widely used to assess alterations in depression-like behaviour in animals. The time spent by the animal as immobile in water represents the depression-like behaviour.[78]

Procedure

The test was performed according to the method described by Porsolt et al., 1977, with slight modifications. Animals were forced to swim in a glass cylinder (20 cm height, 14 cm diameter) containing 10 cm depth of water at 25^{o} c. After the initial 2 min acclimatization period, the total duration of immobility was measured during final 4 min of the 6 min test session. Animal were considered to be immobile, when they made no further attempts to escape except the movements necessary to kept their heads above the water. After 6 min, the animals were removed from water, allowed to dry, and returned back to their home cage.[79]

3.5.4. HOLE BOARD TEST

Principle

When a animal is placed on the hole board apparatus, which is elevated to 25 cm from the base, shows anxiety as it is exposed to a new environment, thus showing characteristics head poking behaviour. Decrease in anxiety shows increased exploration of the holes. Whereas increased anxiety shows lower number of head poking.[80]

Procedure

The hole board apparatus consist of a wooden board (40*40cm) placed 25 cm above the ground. It consists of 16 holes which is about 3 cm in diameter, spaced symmetrically in a diamond pattern. Animals were placed on the corner of the apparatus and were observed for the next 5 min for the number of head dipping. A head dipping is counted when the animal introduces its head into any hole of the box up to the level of the ears. The apparatus was thoroughly cleaned between each subject.[81]

3.5.5. TAIL SUSPENSION TEST

Principle

The tail-suspension test (TST) is a widely used assay for screening potential antidepressant drugs. The test is based on the principle that animal subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture.[82]

Procedure

The tail suspension test is another well characterized test for assessing depression-like and anti-depressant like activity. In this test animal were individually suspended by the tail to a horizontal ring –stand bar (distance from floor = 30cm) using adhesive tape (distance from tip of tail = 2cm). Typically animal demonstrated several escape-orientated behaviours interspersed with temporally increasing bouts of immobility. A 6-mins test session was employed, which was videotaped. The parameter recorded was the number of seconds spent immobile.[83]

3.6. ESTIMATION OF BRAIN NEUROTRANSMITTER

3.6.1. Estimation of Serotonin, GABA and Dopamine

Preparation of tissue extracts

Reagents

- HCl – Butanol solution: (0.85 ml of 37% hydrochloric acid in one-litre *n*-butanol)
- Heptane
- M HCl: (0.85 ml conc. HCl up to 100 ml H_2O)

Procedure

At the end of experiment, rats were sacrificed and the whole brain was dissected out. 0.25 g of tissue was weighed and was homogenized in 5 mL HCl–butanol with motor driven Teflon coated homogenizer for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 mL) was removed and added to centrifuge tube containing heptane (2.5 mL) and 0.1 M HCl (0.31 mL). After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase was then taken either for 5-HT or NA and DA assay.

3.6.2. Estimation of dopamine

Reagents

- 0.4 M HCl: 0.34 ml conc. HCl up to 10 mL H_2O
- Sodium acetate buffer (pH 6.9): 0.72 mL of 1 M acetic acid (6 µL of glacial acetic acid up to 1000 µL with distilled water) + 6.84 mL of 0.3 M sodium acetate (0.408 g of sodium acetate in 10 mL distilled water) and volume were made up to 25 mL with distilled water. pH was adjusted with sodium hydroxide solution.
- 5 M sodium hydroxide: 5 g of NaOH pellets dissolved in distilled water and volume was made up to 25 mL with distilled water.
- M Iodine solution (in Ethanol): 1 g of potassium iodide + 0.65 g of iodine dissolved in ethanol and volume was made up to 25 mL.
- Sodium thiosulphate solution: 0.625 g Na_2SO_3 in 2.5 mL H_2O + 22.5 mL 5 M NaOH
- 10 M Acetic acid: 14.25 mL of glacial acetic acid dissolved in distilled water and made up to 25 mL.

Procedure

To 1 mL of aqueous phase, 0.25 mL 0.4 M HCl and 0.5 mL of Sodium acetate buffer (pH 6. 9) were added followed by 0.5 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by the addition of 0.5 mL Na_2SO_3 solution. 0.5 mL Acetic acid was added after 1.5 min. The solution was then heated to 100°C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine. Blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Different concentration of dopamine and nor-adrenaline (1 mg/ml) was used as standard.[84]

3.6.2. Estimation of Serotonin

The serotonin content was estimated by the OPT method

Reagents

O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.4 mL aqueous extract, 1.75 mL of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorimeter. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/mL) at different concentration was used as standard.[85]

3.6.3. Estimation of brain GABA content

Preparation of tissue homogenate

Animals were sacrificed by decapitation and the whole brain was rapidly removed. 0.5 g tissue was weighed and placed in 5 mL of ice-cold TCA (10% w/v). The tissue was then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. The supernatant was used for estimation of GABA content.

Reagents:

- Carbonate-bicarbonate buffer, 0.5 M (pH 9.95): 1.0501 g sodium bicarbonate and 1.3249 g sodium carbonate dissolved in distilled water and made up to 25 ml. pH adjusted to 9.95 if necessary.
- 0.14 M ninhydrin solution: 499 mg ninhydrin dissolved in 0.5 M carbonate-bicarbonate buffer and made up to 20 ml.
- Copper tartarate reagent: 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid.

Procedure:

0.1 mL of tissue homogenate was placed in 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.95), and kept in a water bath at 60°C for 30 min. It was then cooled and treated with 5 mL of copper tartarate reagent. After 10 min fluorescence at 377/455 nm in a spectofluorimeter was recorded. [86]

3.7 *In vivo* antioxidant activity

3.7.1. Estimation of reduced glutathione (GSH)

Requirements

10%TCA

0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate

0.2 M Phosphate buffer, pH 8.0

Principle

DTNB is a disulfide compound, which was reduced by sulphadryl groups present in GSH. This reduction leads to the formation of yellow color, which was measured at 412 nm.

Procedure : To 1 ml of the homogenate, 1 ml of the TCA solution was added and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 ml of supernatant 2 ml of DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then absorbance was read at 412 nm. The amount of glutathione was expressed as µ/mg protein.[87]

3.7.2. Determination of lipid peroxidation

Requirements

- Thiobarbituric acid 0.37 %
- 0.25 N HCl
- 15%TCA

Principle

This assay is based on the reduction of thiobarbituric acid with malonyl dialdehyde which is a formed as a result of polyunsaturated fatty acid oxidation. This reaction leads to the formation of pink colored TBA-MDA complex which is measured at 532 nm.

Procedure:

To 0.1 ml of sample, 2 ml of TBA-TCA-HCl reagent (ratio of 1:1) was added mixed and kept in a water bath for 15 minutes. Afterward the solution was cooled and supernatant was removed and absorbance was measured at 535 nm against reference blank. The level of lipid peroxides was given as nm moles of MDA formed/mg protein.[88]

3.7.3 Estimation of proteins

Requirements

- Alkaline copper reagent
- Solution A: 2% sodium carbonate in 0.1 N NaOH
- Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate .50 ml of solution A was mixed with 1 ml of solution B just before use.
- Folin's phenol reagent (commercial reagent, 1:2 dilutions),
- Bovine serum albumin (BSA).

Principle

This method is a combination of both Folin-ciocalteau and biuret reaction which involves two steps

Step: 1 Protein binds with copper in alkaline medium and reduces it to Cu++.

Step: 2 The cu++ formed catalyzes the oxidation reaction of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum ,which leads to the formation of blue color and absorbance was measured at 640 nm.

Procedure:

To 0.1 ml of the homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand at the room temperature for 10 minutes. To this 0.5 ml of folin'reagent was added. After 20 minutes, the color developed was measured at 640 nm. The level of protein present was expressed as mg/g/ tissue or mg/dl.[89]

3.8. HISTOPATHOLOGY TECHNIQUES

The brain was collected and washed under saline and preserved in 10% buffered formalin. The tissues were trimmed into sections and were subjected to prepare paraffin blocks. 5 microns thickness sections were cut and stained with haematoxylin and eosin (H & E) and observed under the microscope.

3.9. STATISTICAL ANALYSIS

The statistical analysis was carried out by using PRISM version 5 software. The data's of all parameters were analysed by means of one way ANOVA followed by Dunnett's test. The results were expressed as mean ±SEM.

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4. RESULTS

4.1. EXTRACTIVE YIELD

Percentage Yield of AEBP

Coarsely powdered whole plant of *Barleria prionitis* were extracted with water using maceration technique and the percentage yield was found to be 12% w/w.

4.2. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table 6 : Phytochemical analysis of AEBP

S.NO	PHYTOCHEMICAL CONSTITUENTS	AEBP
1	Alkaloids	Negative
2	Flavonoids	Present
3	Steroids	Negative
4	Triterpenoids	Present
5	Reducing sugars	Negative
6	Tannins	Present
7	Glycosides	Present
8	Protein and Amino acids	Negative
9	Saponins	Present
10	Phenols	Present

4.3. SCREENING OF ANTIPARKINSONIAN ACTIVITY OF AEBP: [Rotenone Model]

4.3.1. ROTA ROD TEST:

Table 7 : Effect of AEBP on muscle grip strength

Groups	Time spent on Rota rod (sec)		
	Day 3	Day 5	Day 7
Vehicle control	129±1.15	129±1.44	130±1
ROTENONE	69.667±1.20***	60±1.52***	43.333±9.98***
ROTENONE + Levodopa/benzerazide	58±2.08***	81.333±1.20***	118.67±6.75***
ROTENONE + AEBP (200 mg/kg)	47.667±2.603***	62.333±0.88***	95±7.48***
ROTENONE + AEBP (400 mg/kg)	53±2.08***	76.333±6.634***	111.33±3.52***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. A $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

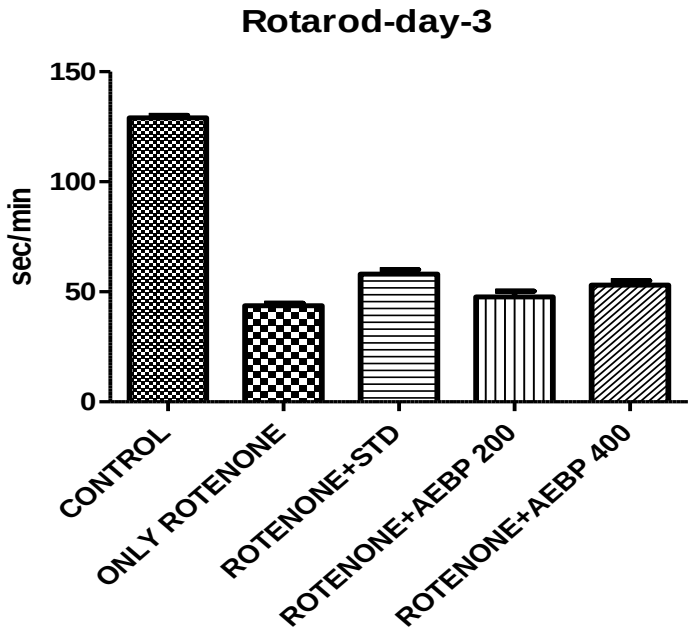


Figure 6 : Effect of AEBP on muscle grip strength- day 3

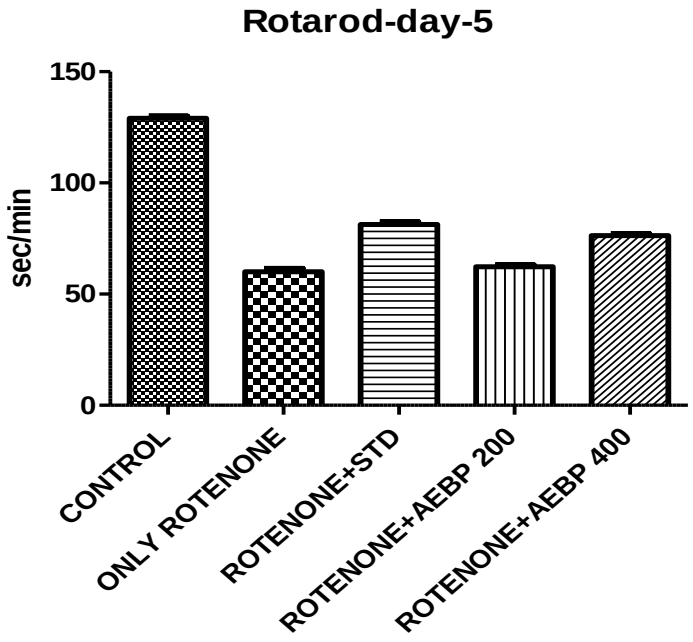


Figure 7 : Effect of AEBP on muscle grip strength- day 5

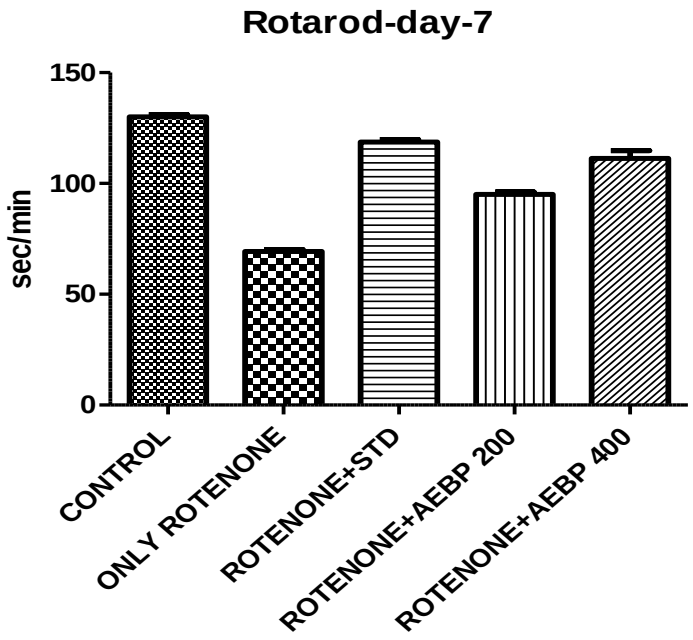


Figure 8 : Effect of AEBP on muscle grip strength- day 7

4.3.2. ACTOPHOTOMETER TEST:-

Table 8 :- Effect of AEBP on Spontaneous locomotor activity

Groups	Locomotive score (sec)		
	Day 3	Day 5	Day 7
Vehicle control	392.33±4.46	412±2.08	396±1.15
ROTENONE	195.33±8.04***	173.33±1.85***	157.67±1.42***
ROTENONE + Levodopa/benzerazide	176±1.16***	228.33±1.45***	258.33±2.42***
ROTENONE + AEBP (200 mg/kg)	169±1.73***	198±5.13***	224.33±2.90***
ROTENONE +AEBP (400 mg/kg)	178.33±2.02***	212.33±2.17***	246.33±2.18***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

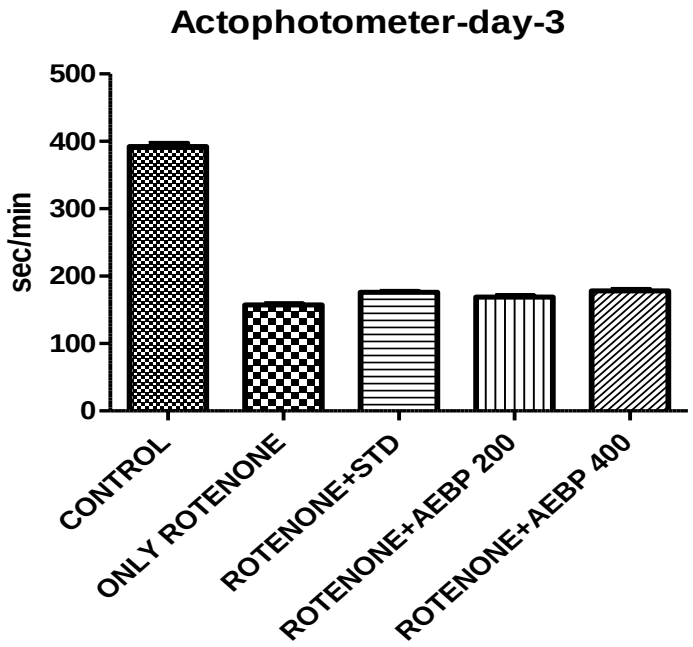


Figure 9 : Effect of AEBP on locomotor activity - day 3

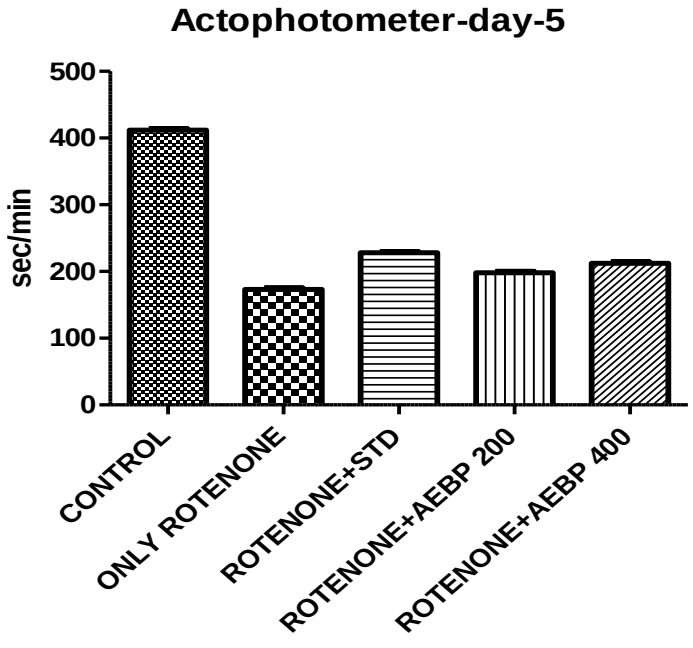


Figure 10 : Effect of AEBP on locomotor activity - day 5

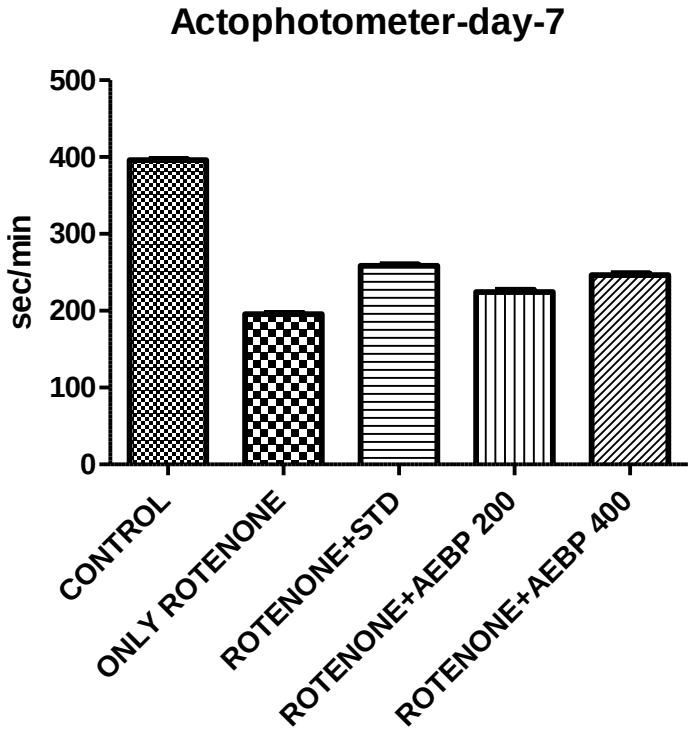


Figure 11 : Effect of AEBP on locomotor activity - day 7

4.3.3. FORCED SWIM TEST:-

Table 9 : Effect of AEBP on depression

Groups	Immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	55±4.22	52±1.23	53±1.94
ROTENONE	128.67±0.85***	130.33±2.40***	132.67±1.45***
ROTENONE + Levodopa/benzerazide	125.33±1.36***	107.33±2.33***	90±1***
ROTENONE + AEBP (200 mg/kg)	133.33±2.60***	125.33±4.45***	121.33±3.09***
ROTENONE +AEBP (400 mg/kg)	135.67±0.09***	119.67±1.21***	99.667±1.22***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

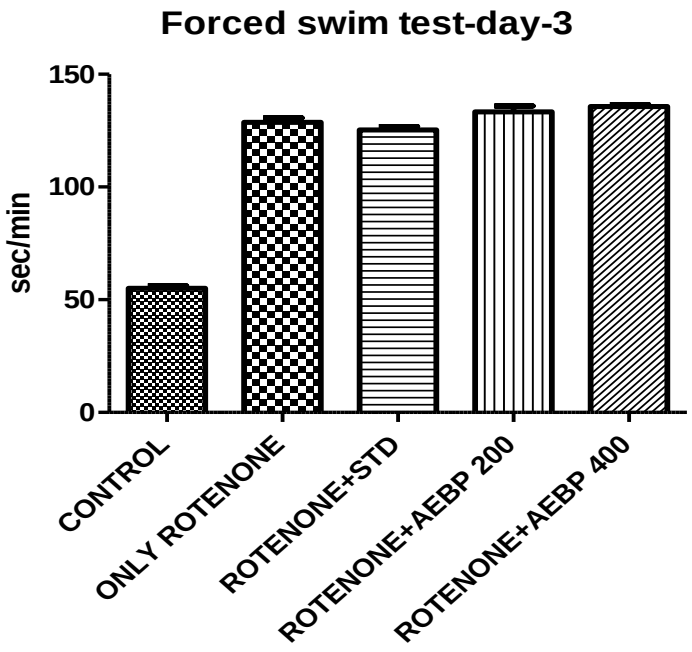


Figure 12 : Effect of AEBP on depression by forced swim test- day 3

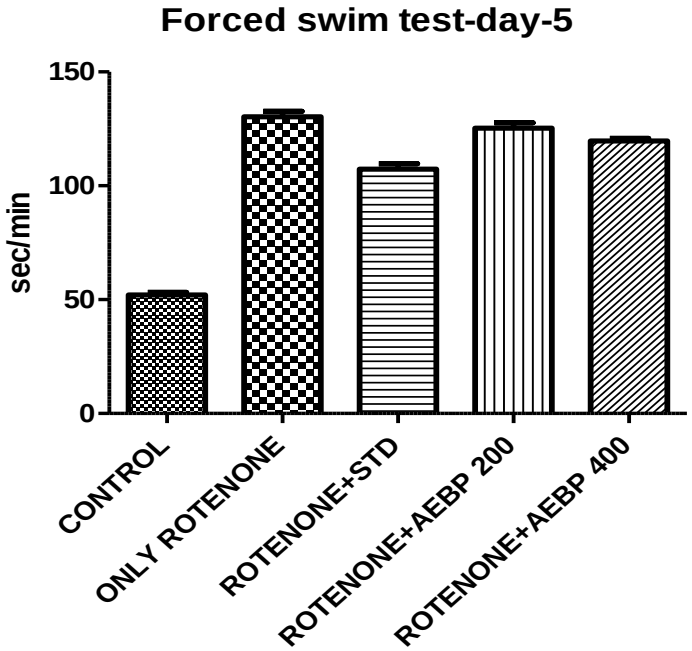


Figure 13 : Effect of AEBP on depression by forced swim test- day 5

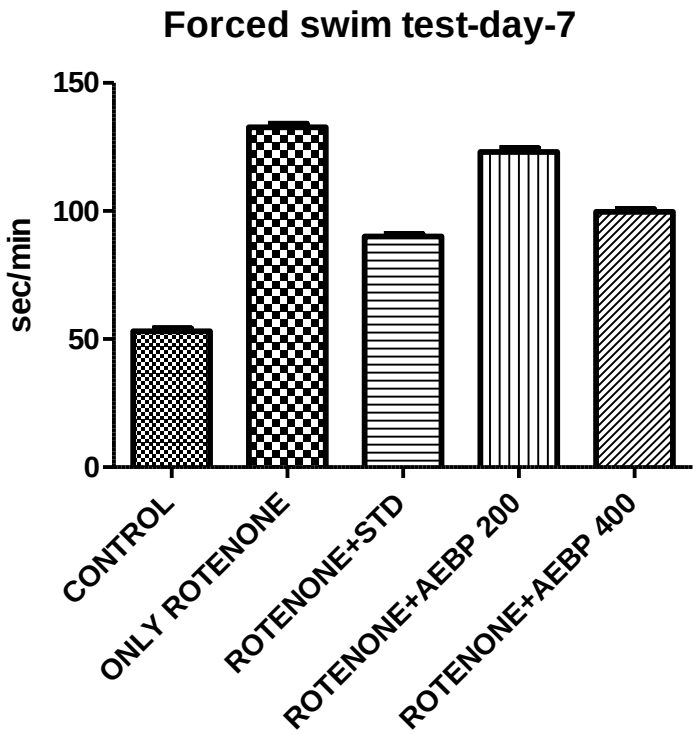


Figure 14 : Effect of AEBP on depression by forced swim test- day 7

4.3.4. TAIL SUSPENSION TEST:-

Table 10 : Effect of AEBP on depression

Groups	Immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	58±0.89	54.333±1.45	55±4.46
ROTENONE	132.67±1.76***	135±1.52***	136±2.32***
ROTENONE + Levodopa/benzerazide	127.33±1.85***	108.33±1.12***	92±2.30***
ROTENONE + AEBP (200 mg/kg)	136.67±3.56***	126.67±0.75***	124±1.97***
ROTENONE +AEBP (400 mg/kg)	132±1.33***	122.67±3.71***	102.67±2.78***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

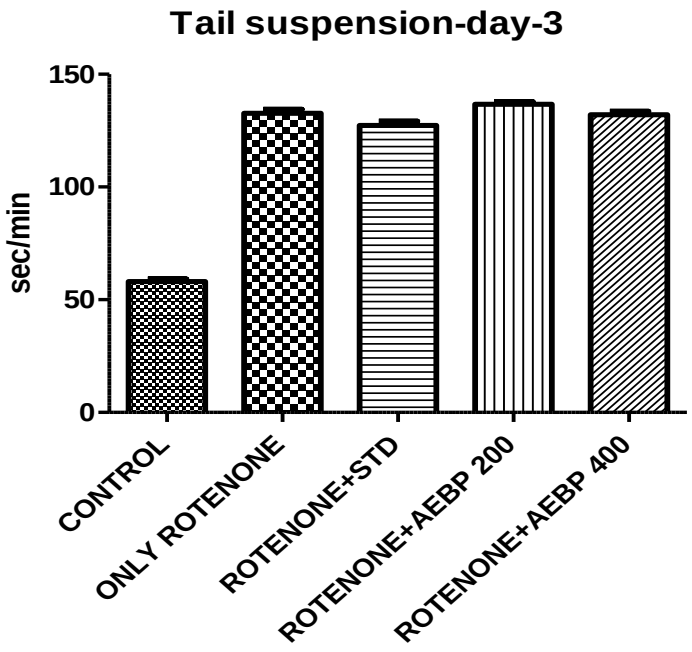


Figure 15 : Effect of AEBP on depression by tail suspension test-day 3

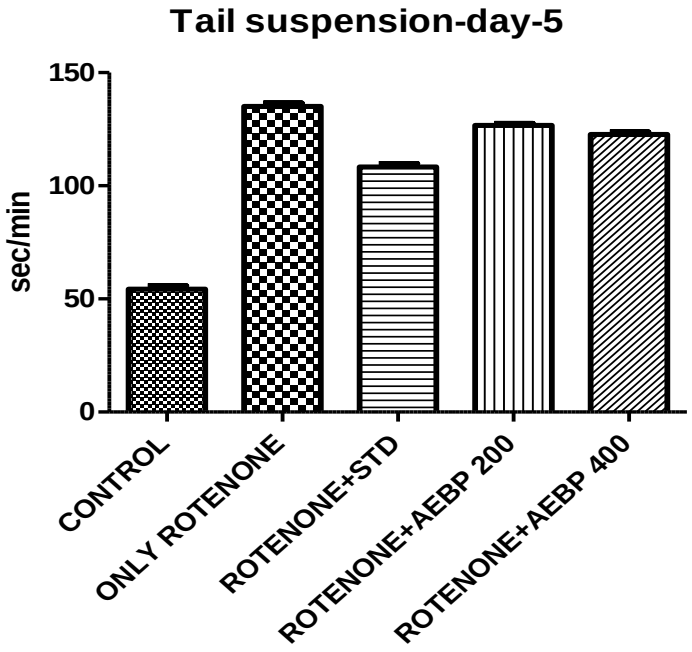


Figure 16 : Effect of AEBP on depression by tail suspension test-day 5

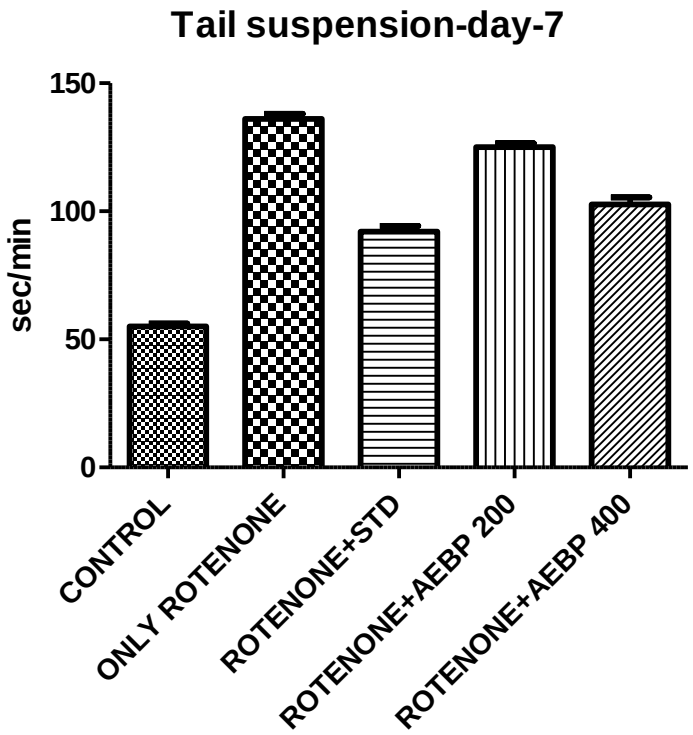


Figure 17 : Effect of AEBP on depression by tail suspension test-day 7

4.3.5. HOLE BOARD TEST:-

Table 11 : Effect of AEBP on alertness

Groups	No. of head dippings		
	Day 3	**Day 5**	**Day 7**
Vehicle control	32±3.06	32±4.52	29±2.42
ROTENONE	21.667±1.76**	20.333±2.95***	14±2.51***
ROTENONE + Levodopa/benzerazide	23±1**	23±1.73**	27.333±0.66ns
ROTENONE + AEBP (200 mg/kg)	13.667±1.76***	12.667±0.43***	18.667±3.01***
ROTENONE +AEBP (400 mg/kg)	16±0.08***	20.333±4.03***	23.333±0.31***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

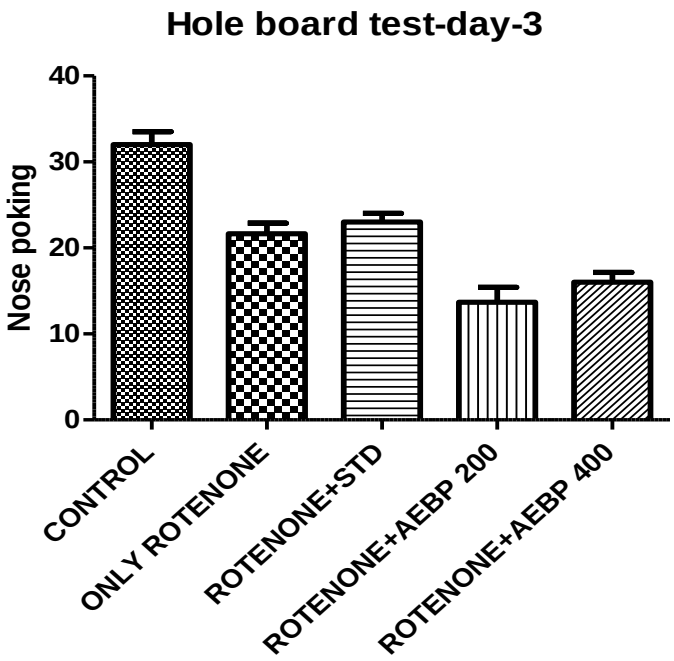


Figure 18 : Effect of AEBP on depression by hole board test-day 3

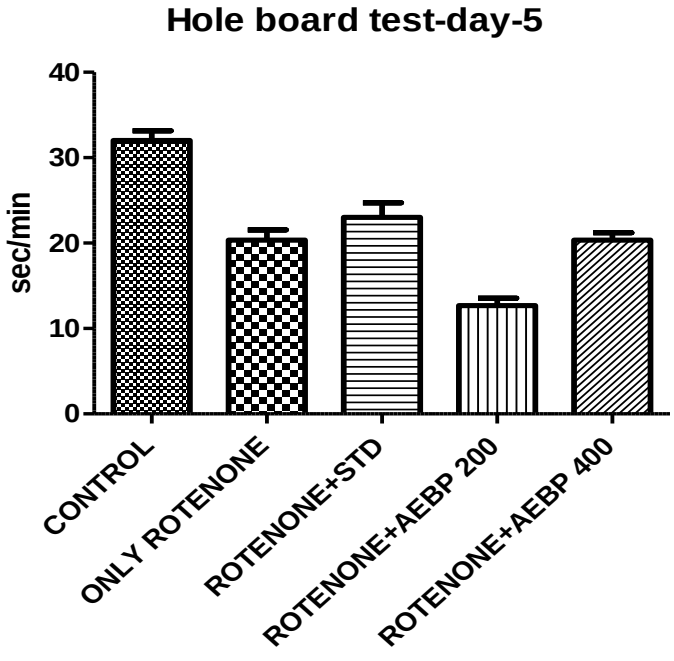


Figure 19 : Effect of AEBP on depression by hole board test-day 5

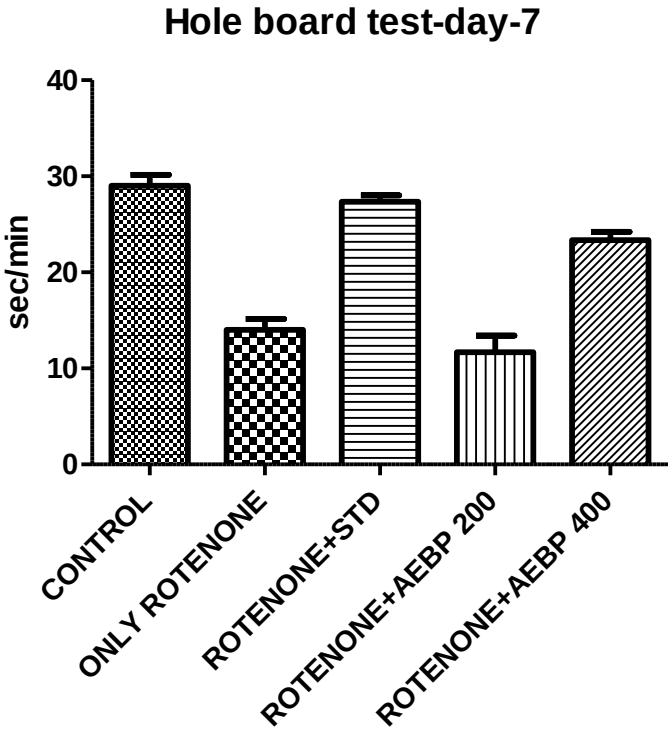


Figure 20 : Effect of AEBP on depression by hole board test-day 7

4.4. ESTIMATION OF TOTAL PROTEIN

Table 12 : Effect of AEBP on total protein in brain tissue

Groups	TOTAL PROTEIN (mg/100mg of tissue)
Vehicle control	5.43±0.16
ROTENONE	2.17±0.01***
ROTENONE+ Levodopa/benzerazide	4.09±0.08***
ROTENONE + AEBP (200 mg/kg)	2.8333±0.11***
ROTENONE +AEBP (400 mg/kg)	3.8733±0.04***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

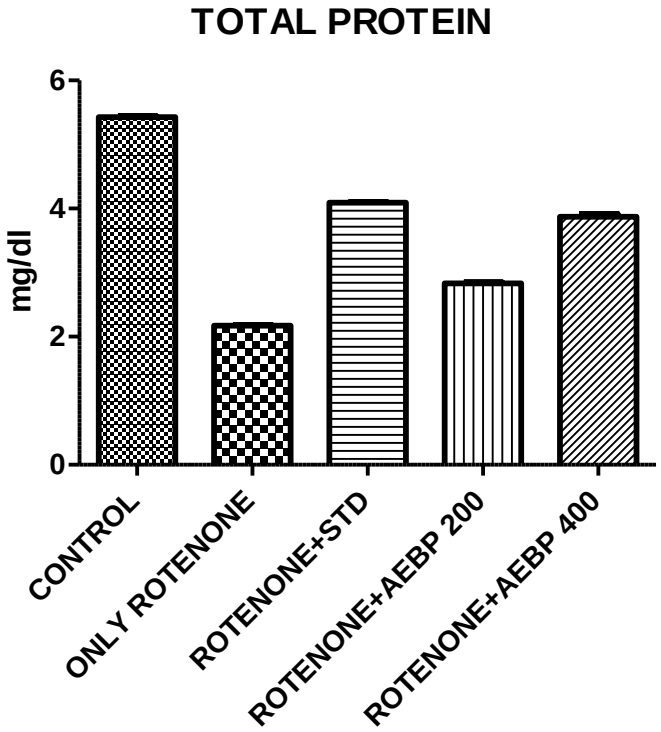


Figure 21 : Effect of AEBP on total protein in brain tissue

4.5. *IN VIVO* ANTIOXIDANT STUDY

Table 13 : Effect of AEBP on *IN VIVO* antioxidants of brain of ROTENONE induced parkinsonic mice

Groups	GSH (Glutathione µg/mg protein)	LPO (nmol of MDA/mg protein)
Vehicle control	5.0733±0.043	3.7267±0.012
ROTENONE	2.1267±0.027***	4.93±0.011***
ROTENONE + Levodopa/benzerazide	4.39±0.026***	3.9217±0.003***
ROTENONE + AEBP (200 mg/kg)	2.9233±0.035***	4.1167±0.017***
ROTENONE +AEBP (400 mg/kg)	3.9833±0.020***	3.1067±0.008***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

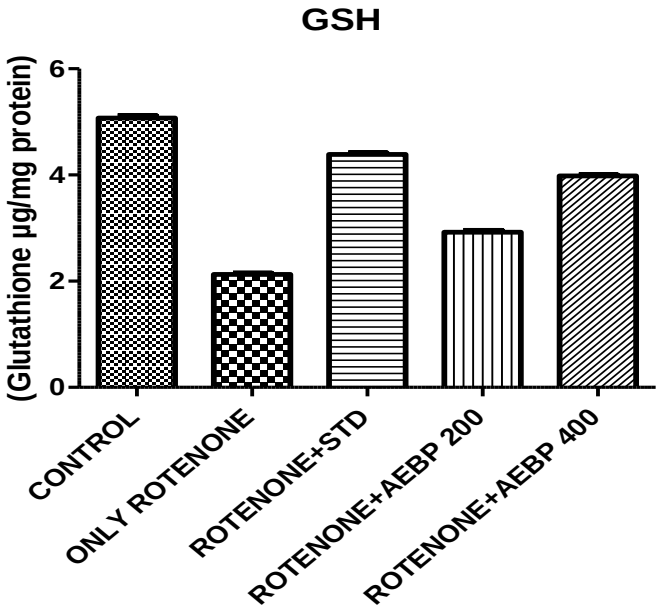


Figure 22 : Effect of AEBP on GSH in brain tissue

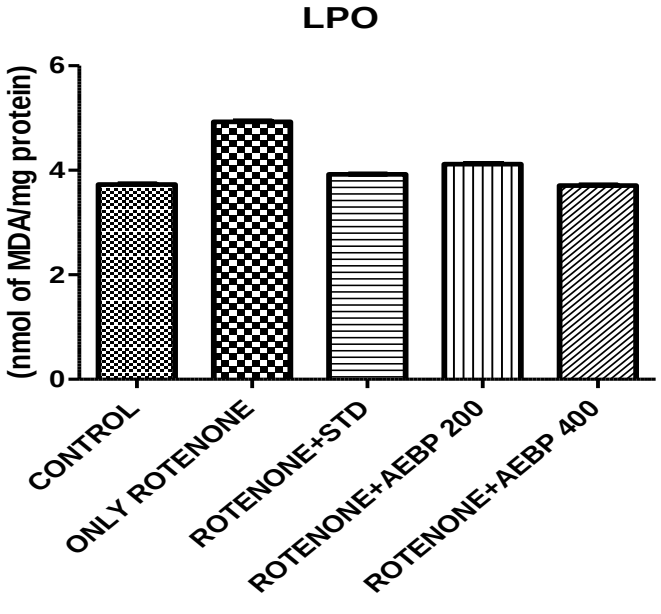


Figure 23 : Effect of AEBP on LPO in brain tissue

4.6. ESTIMATION OF BRAIN NEUROTRANSMITTERS

Table 14 : Effect of AEBP on brain neurotransmitters of ROTENONE induced parkinsonic rats

Groups	DOPAMINE(ng/g tissue)	SEROTONINE(ng/g tissue)	GABA(ng/g tissue)
vehicle control	373.33±0.06	379.33±0.88	213.67±2.31
ROTENONE	174.33±1.30***	189.67±1.16***	125.33±1.11***
ROTENONE + Levodopa/benzerazide	292.67±1.66***	286.33±1.10***	154.33±1.22***
ROTENONE + AEBP (200 mg/kg)	187.67±2.70***	205.67±0.42***	142.33±0.51***
ROTENONE +AEBP (400 mg/kg)	228.67±1.32***	220.33±0.11***	155.33±1.76***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

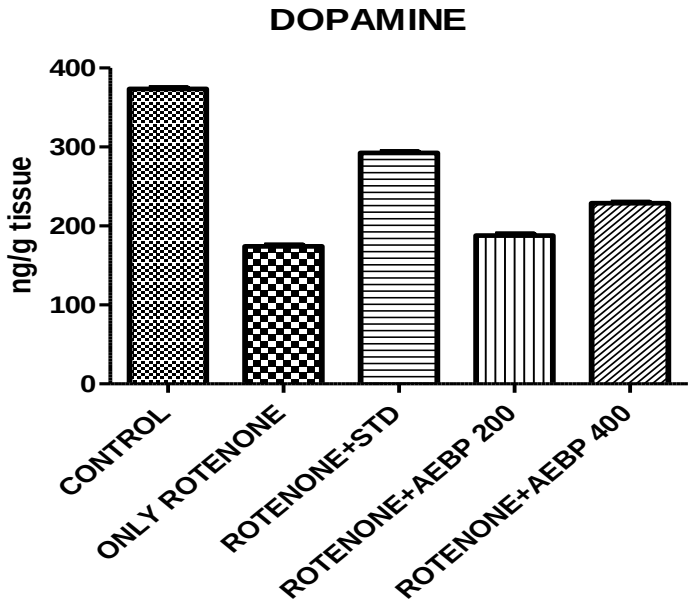


Figure 24 : Effect of AEBP on dopamine in brain tissue

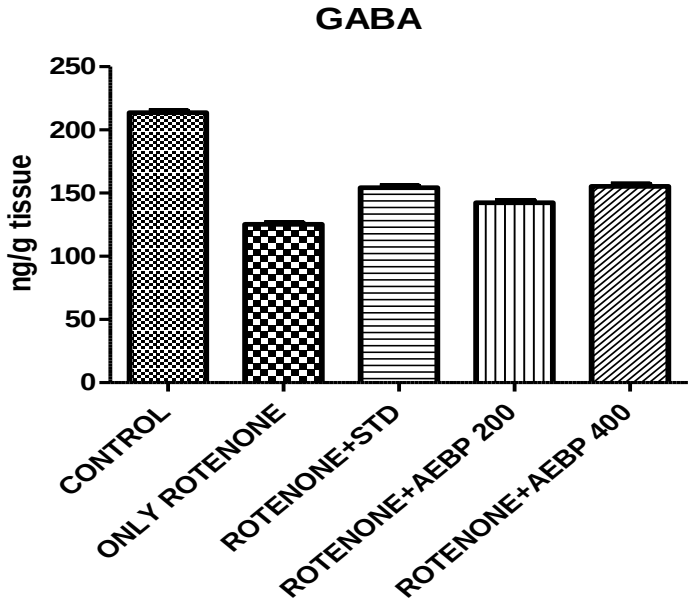


Figure 25 : Effect of AEBP on GABA in brain tissue

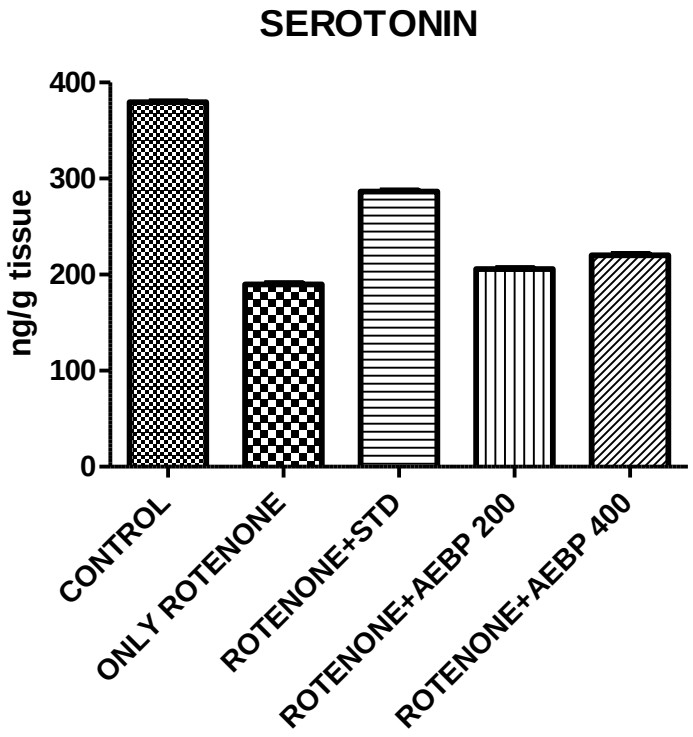


Figure 26 : Effect of AEBP on serotonin in brain tissue

4.7. SCREENING OF ANTIPARKINSONIAN ACTIVITY OF AEBP:- [MPTP MODEL]

4.7.1. ROTA ROD TEST:-

Table 15 : Effect of AEBP on muscle grip strength

Groups	Time spent on Rota rod (sec)		
	Day 3	Day 5	Day 7
Vehicle control	178±0.57	179±1.48	178.67±0.95
MPTP	74.667±1.33***	62±0.26***	61.333±0.18***
MPTP+ Levodopa/benzerazide	59±2.02***	84.667±1.76***	120.67±0.59***
MPTP + AEBP (200 mg/kg)	47±1.52***	67±0.64***	94.667±0.72***
MPTP + AEBP (400 mg/kg)	53.333±2.40***	81±2.08***	111.67±0.14***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

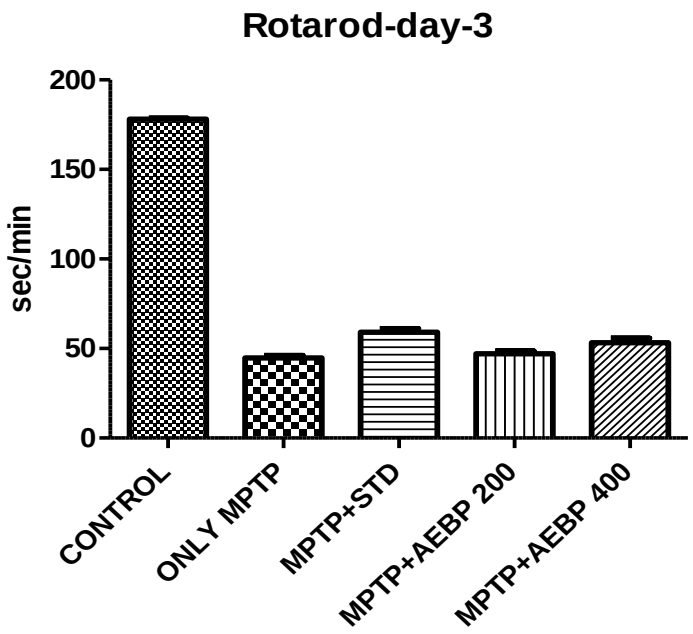


Figure 27 : Effect of AEBP on muscle grip strength- day 3

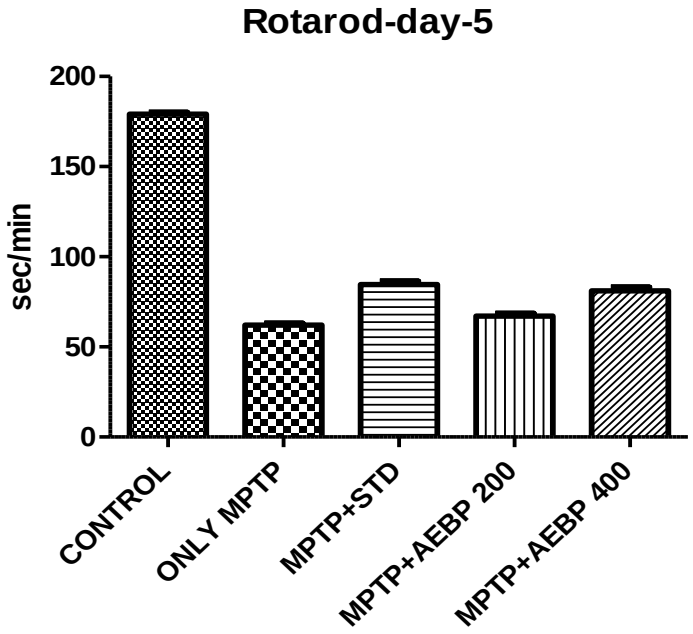


Figure 28 : Effect of AEBP on muscle grip strength- day 5

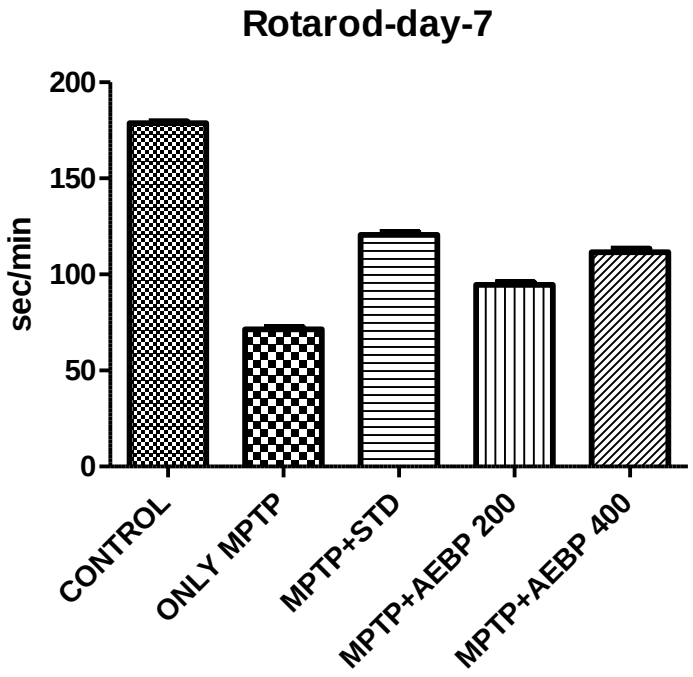


Figure 29 : Effect of AEBP on muscle grip strength- day 7

4.7.2. ACTOPHOTOMETER TEST:

Table 16 :- Effect of AEBP on Spontaneous locomotor activity

Groups	Locomotive score (sec)		
	Day 3	Day 5	Day 7
Vehicle control	384±3.60	394±1.15	386.33±1.56
MPTP	186±2.30***	162.67±2.02***	145.33±0.23***
MPTP+ Levodopa/benzerazide	166.33±2.60***	217.33±0.02***	246.33±0.77***
MPTP + AEBP (200 mg/kg)	155.33±1.48***	184.67±0.91***	215.67±0.56***
MPTP +AEBP (400 mg/kg)	162±1.55***	198±1.12***	236.67±1.85***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

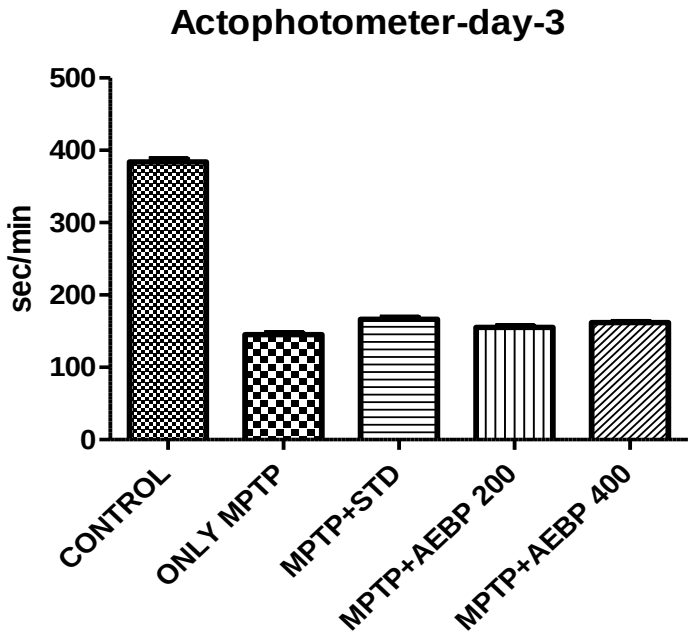


Figure 30 : Effect of AEBP on locomotor activity - day 3

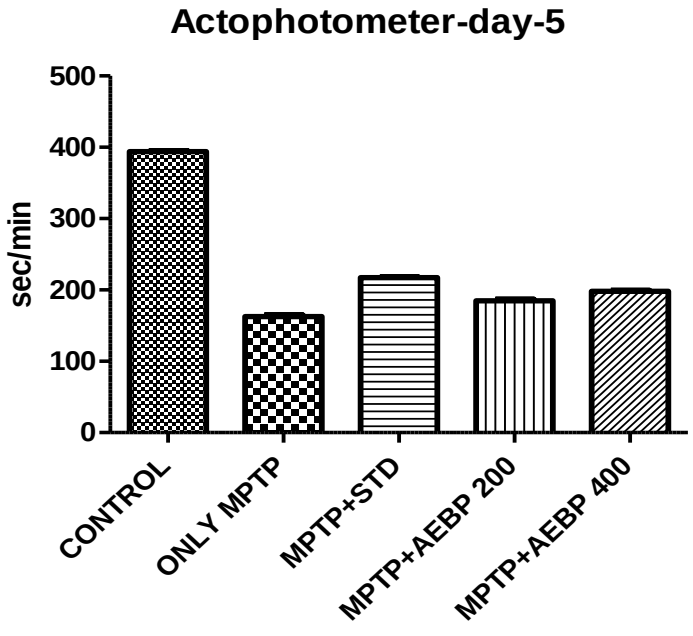


Figure 31 : Effect of AEBP on locomotor activity - day 5

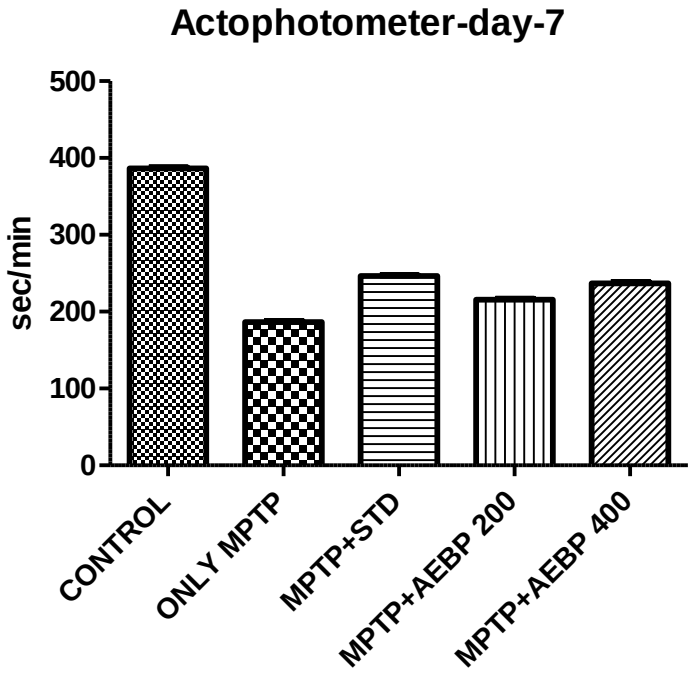


Figure 32 : Effect of AEBP on locomotor activity - day 7

4.7.3. FORCED SWIM TEST:

Table 17 : Effect of AEBP on depression

Groups	Total immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	52.333±2.60	47.667±0.93	48.667±1.33
MPTP	125±0.73***	130.33±1.76***	131.33±2.37***
MPTP+ Levodopa/benzerazide	117.67±0.06***	101±1.15***	85.333±1.78***
MPTP + AEBP (200 mg/kg)	127.67±2.85***	120.67±0.89***	118.67±2.27***
MPTP +AEBP (400 mg/kg)	127±1.01***	116.33±3.74***	93.333±0.15***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

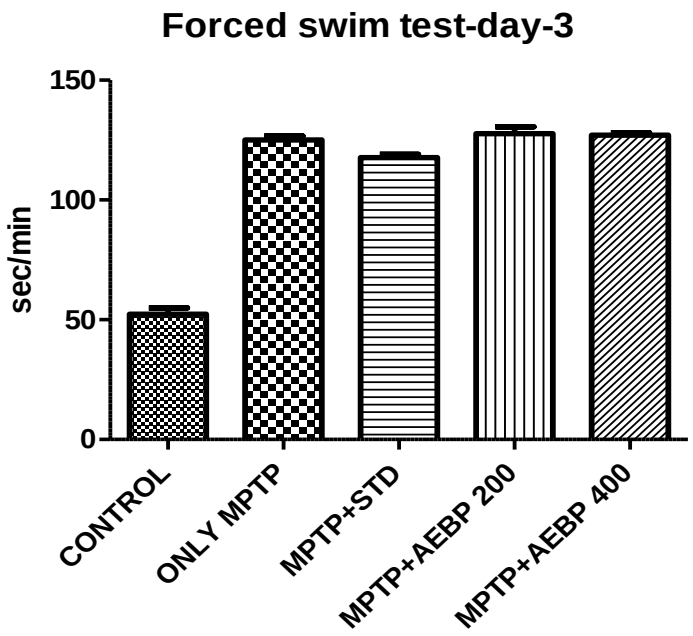


Figure 33 : Effect of AEBP on depression by forced swim test- day 3

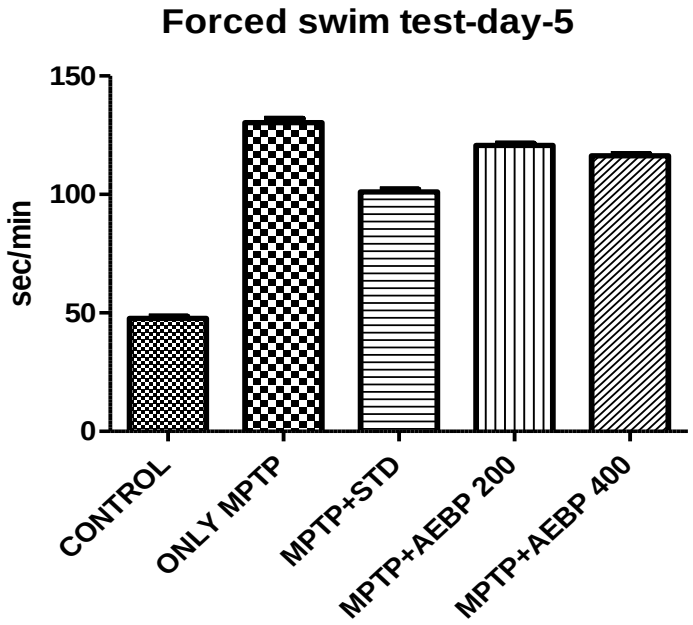


Figure 34 : Effect of AEBP on depression by forced swim test- day 5

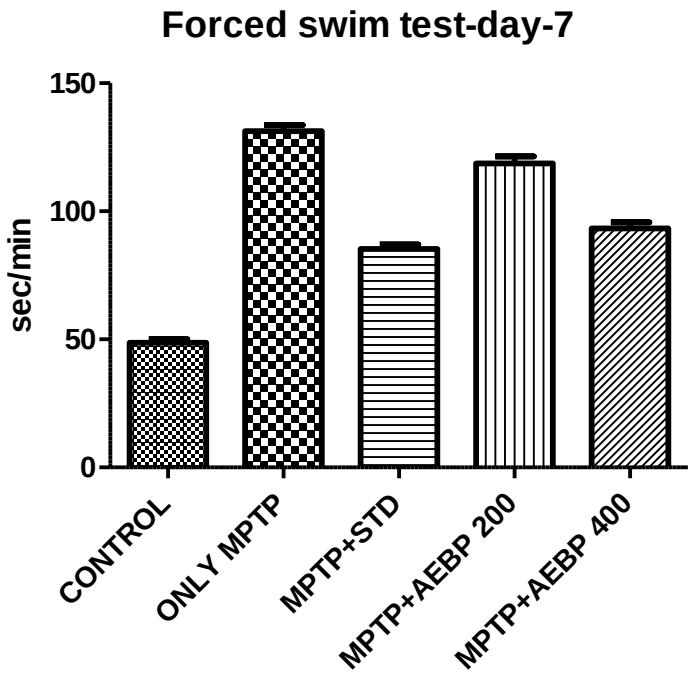


Figure 35 : Effect of AEBP on depression by forced swim test- day 7

4.7.4. TAIL SUSPENSION TEST :-

Table 18 : Effect of AEBP on depression

Groups	Immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	54.667±1.29	52.667±1.85	53±1.52
MPTP	129.67±3.33***	132.67±0.04***	131±2.47***
MPTP+ Levodopa/benzerazide	123±2.08***	103.67±1.66***	87.667±0.18***
MPTP + AEBP (200 mg/kg)	132.67±0.36***	123.33±1.45***	121.33±4.93***
MPTP +AEBP (400 mg/kg)	128.33±2.12***	118.33±1.48***	96±0.83***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

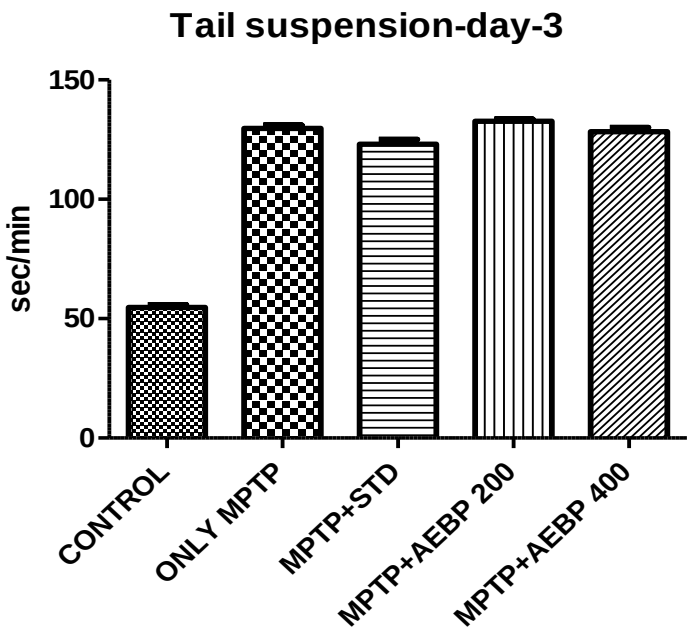


Figure 36 : Effect of AEBP on depression by tail suspension test- day 3

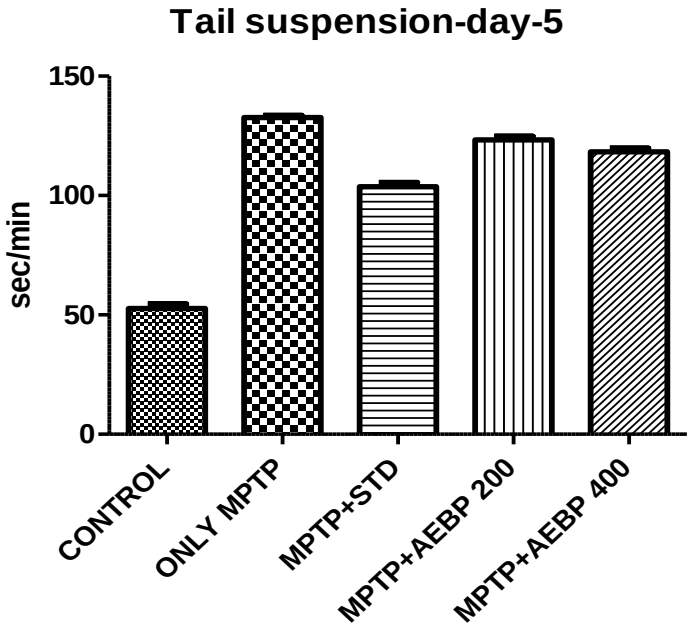


Figure 37 : Effect of AEBP on depression by tail suspension test- day 5

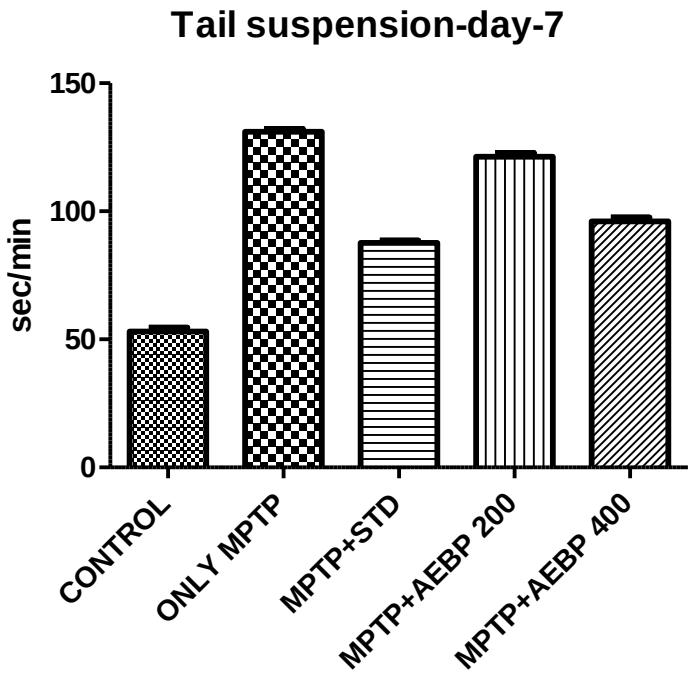


Figure 38 : Effect of AEBP on depression by tail suspension test- day 7

4.7.5. HOLE BOARD TEST:-

Table 19 : Effect of AEBP on alertness

Groups	No. of head dippings		
	Day 3	Day 5	Day 7
Vehicle control	36±1.31	36±0.14	33.667±2.46
MPTP	22±3.68***	22.333±1.79**	18.333±4.82***
MPTP+ Levodopa/benzerazide	23±1.67***	24±2.09**	31.333±0.88ns
MPTP + AEBP (200 mg/kg)	18.333±3.36***	18±1.73***	21.333±0.91***
MPTP +AEBP (400 mg/kg)	23.333±4.55***	24.333±2.52**	27.333±1.20*

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

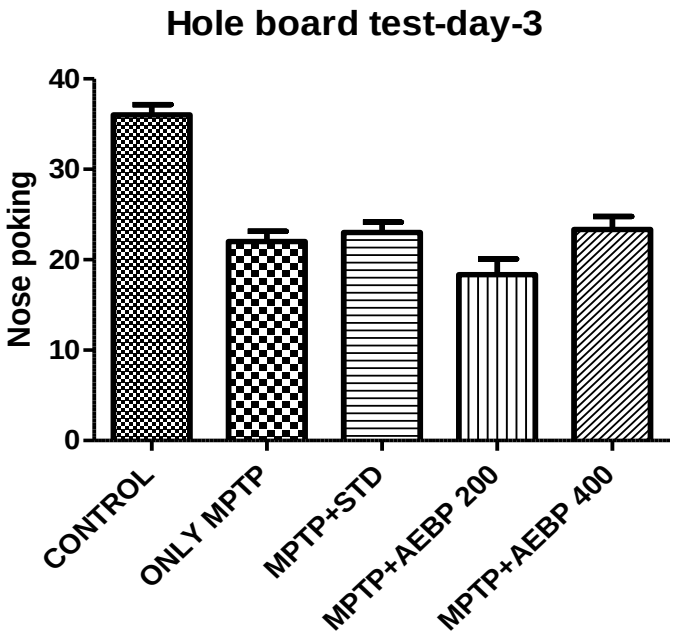


Figure 39 : Effect of AEBP on depression by hole board test-day 3

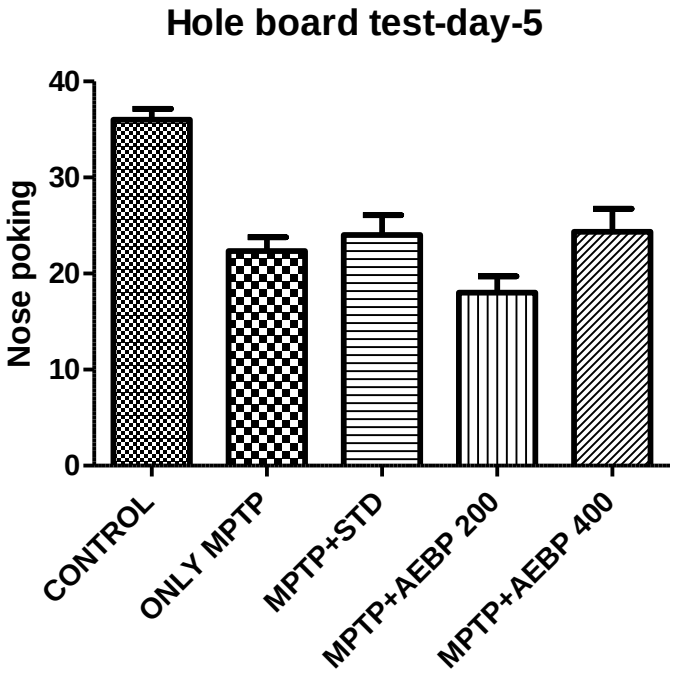


Figure 40 : Effect of AEBP on depression by hole board test-day 5

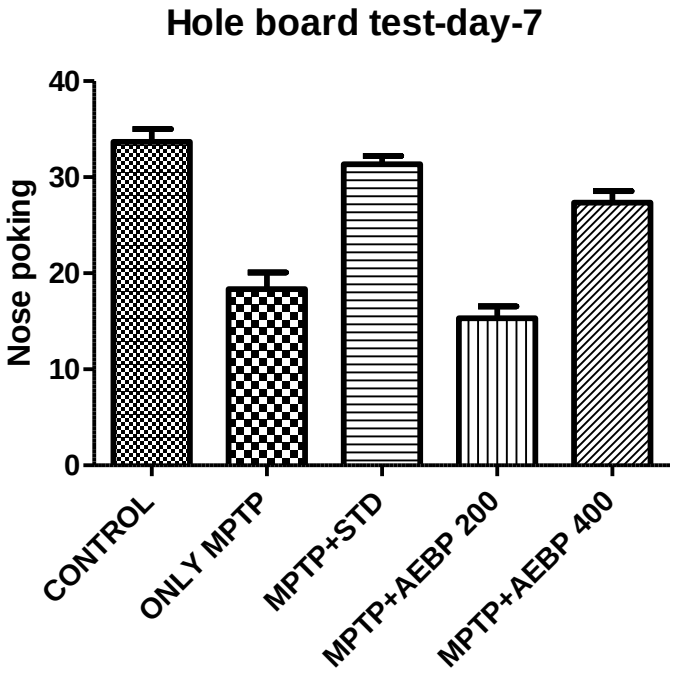


Figure 41 : Effect of AEBP on depression by hole board test-day 7

4.8. ESTIMATION OF TOTAL PROTEIN

Table 20 : Effect of AEBP on total protein in brain tissue

Groups	TOTAL PROTEIN (mg/100mg of tissue)
Vehicle control	3.3533±0.0202
MPTP	4.4533±0.0133***
MPTP+ Levodopa/benzerazide	3.8267±0.0088***
MPTP + AEBP (200 mg/kg)	4.0833±0.0437***
MPTP +AEBP (400 mg/kg)	3.6067±0.0352***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

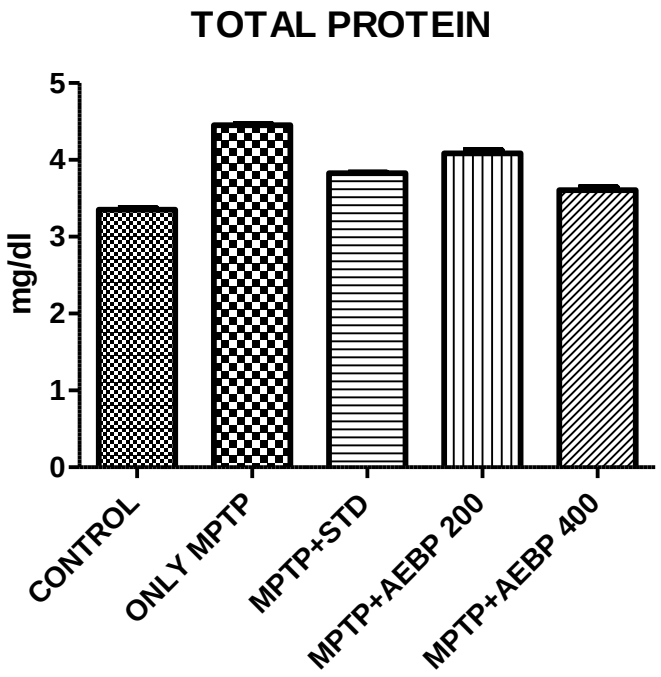


Figure 42 : Effect of AEBP on total protein in brain tissue

4.9. *IN VIVO* ANTIOXIDANT STUDY

Table 21 : Effect of AEBP on *IN VIVO* antioxidants of brain of MPTP induced parkinsonic mice.

Groups	GSH (Glutathione µg/mg protein)	LPO (nmol of MDA/mg protein)
Vehicle control	6.4933±0.0014	6.1547±0.0588
MPTP	2.34±0.0556***	11.436±0.0067***
MPTP+ Levodopa/benzerazide	4.225±0.0565***	7.2437±0.0014***
MPTP + AEBP (200 mg/kg)	3.2367±0.0638***	9.055±0.0062***
MPTP +AEBP (400 mg/kg)	4.1457±0.0560***	7.5247±0.0075***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. [a]P<0.001 denotes comparison of parkinsonic control with vehicle control and ns- non significant [*]P<0.05, [**]P<0.01, and [***]P<0.001 denotes comparison of all groups with parkinsonic control.

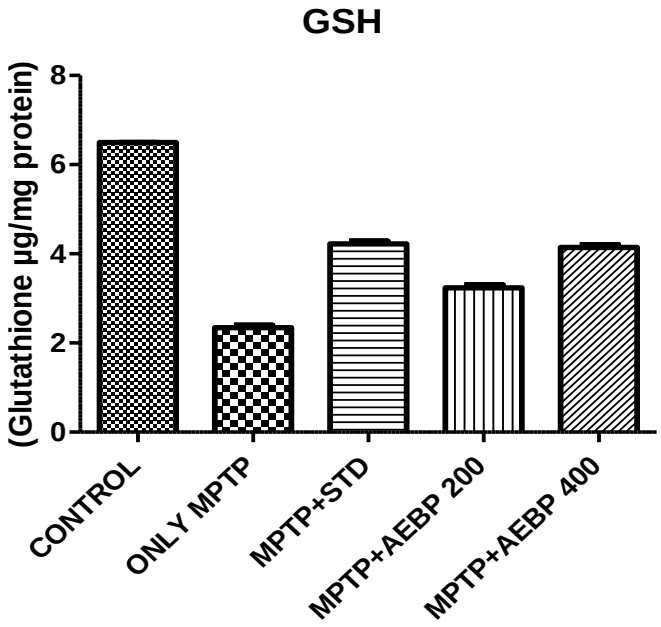


Figure 43 : Effect of AEBP on GSH in brain tissue

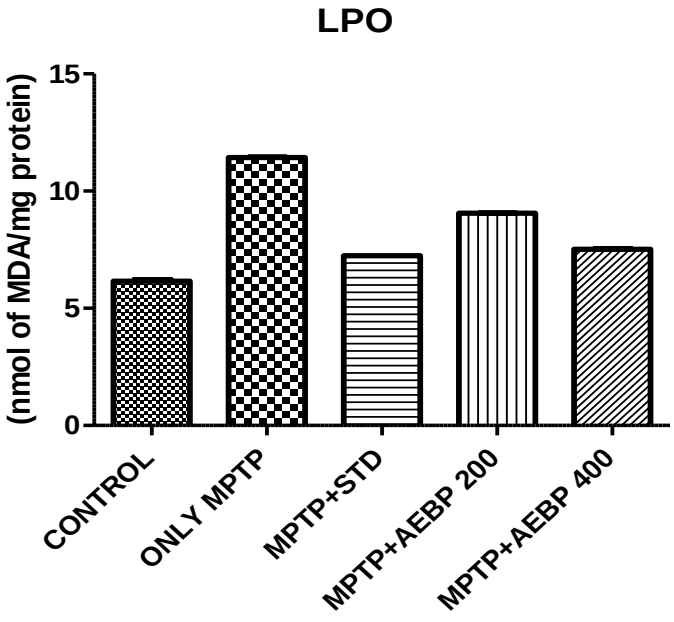


Figure 44 : Effect of AEBP on LPO in brain tissue

4.10. ESTIMATION OF BRAIN NEUROTRANSMITTERS

Table 22 : Effect of AEBP on brain neurotransmitters of MPTP induced parkinsonic mice

Groups	DOPAMINE (ng/g tissue)	SEROTONINE(ng/g tissue)	GABA(ng/g tissue)
Vehicle control	367.67±11.28	357.67±2.84	203±1.52
MPTP	188±1.41***	174.33±3.45***	109.33±2.49***
MPTP+ Levodopa/benzerazide	287±5.50***	273±2.08***	142±0.03***
MPTP + AEBP (200 mg/kg)	198.33±4.33***	192.67±0.58***	128.33±1.85***
MPTP +AEBP (400 mg/kg)	217±2.30***	206±4.46***	138.67±1.33***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way Anova followed by Dunnett's test was performed. $^{a}P<0.001$ denotes comparison of parkinsonic control with vehicle control and ns- non significant $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ denotes comparison of all groups with parkinsonic control.

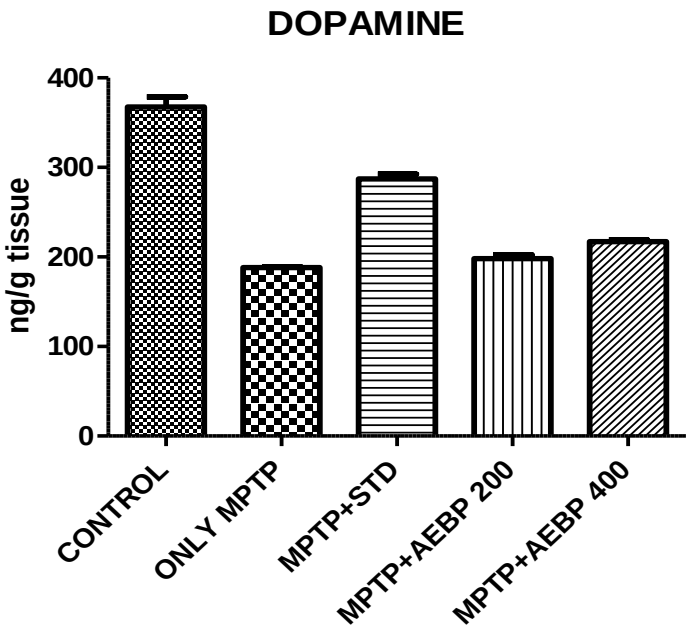


Figure 45 : Effect of AEBP on dopamine in brain tissue

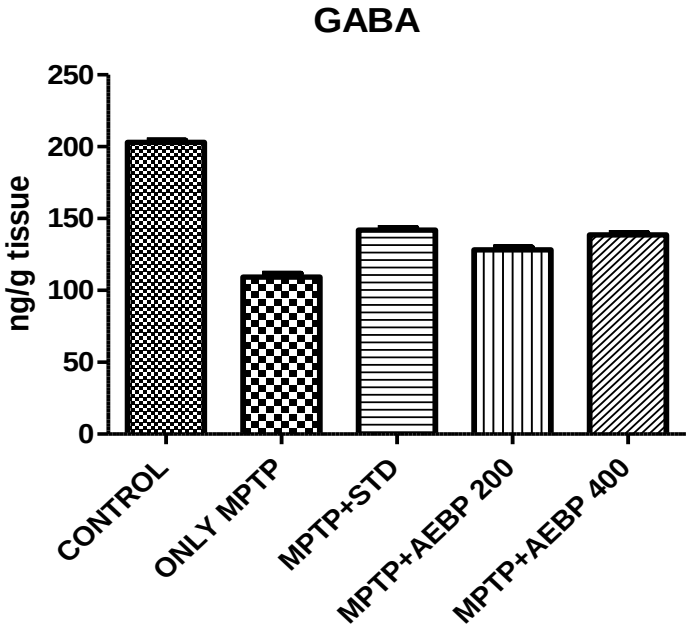


Figure 46 : Effect of AEBP on GABA in brain tissue

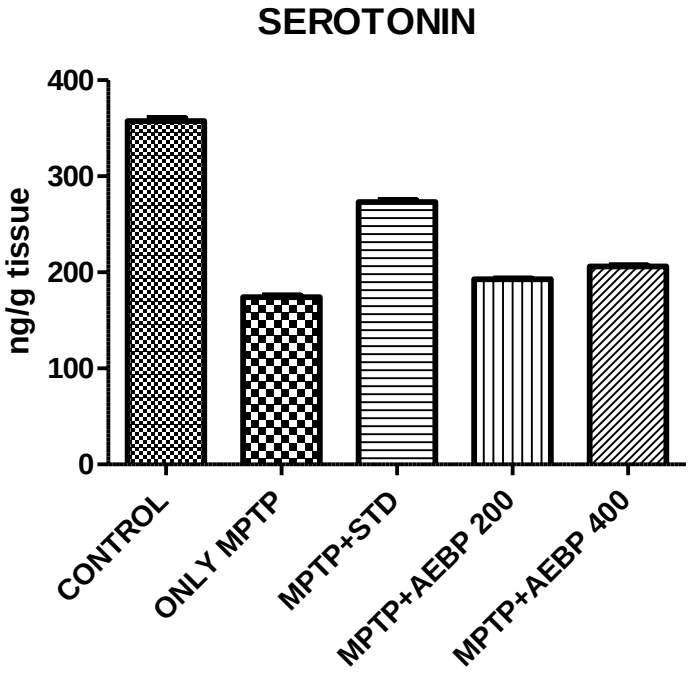
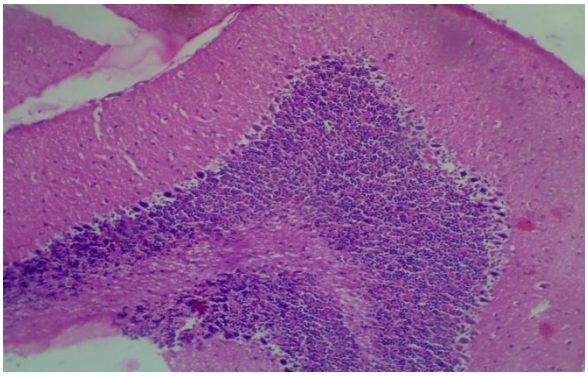
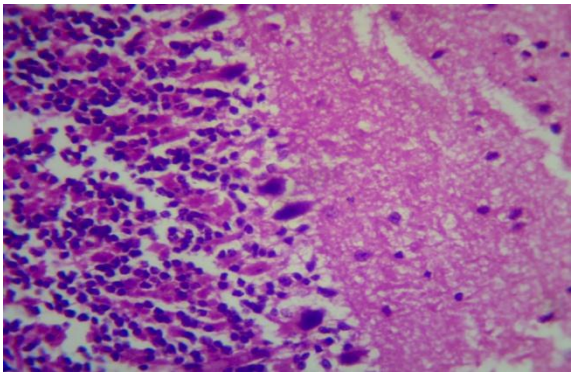


Figure 47 : Effect of AEBP on serotonin in brain tissue

HISTOPATHOLOGICAL EVALUATION MPTP INDUCED PARKINSONISM

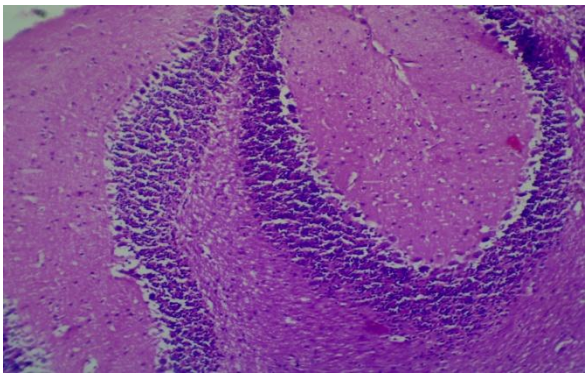


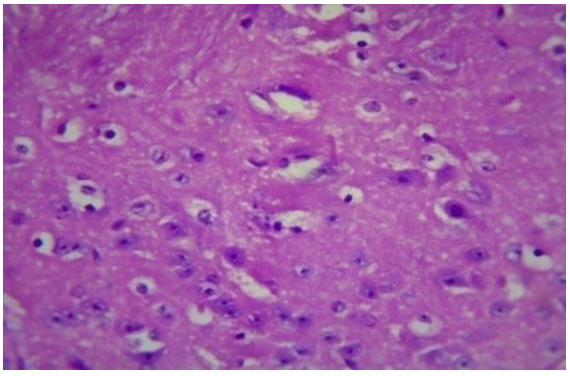


10X **40X**

VEHICLE CONTROL

From the results the Mice brain cerebral cortex shows normal morphology. And shows neurons are normal in number and morphology surrounding parenchyma shows normal histology. The molecular purkinjiec layer shows normal. There is no evidence of neuronal degeneration/inflammation.

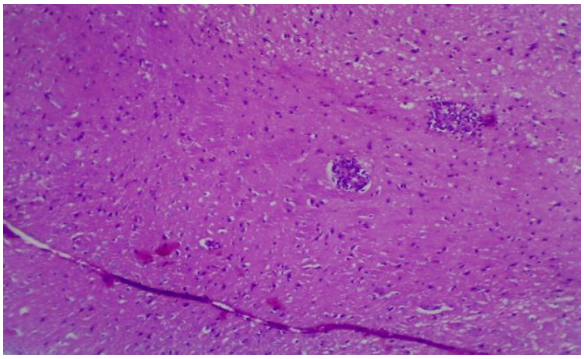
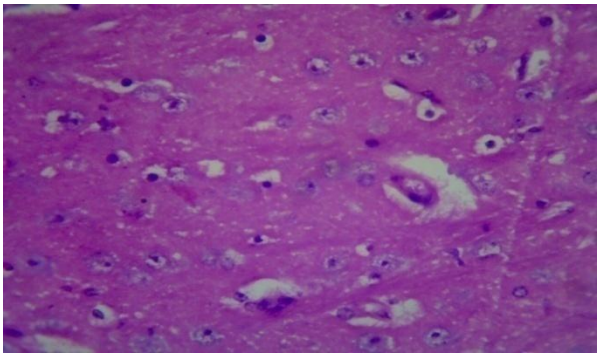




10X **40X**

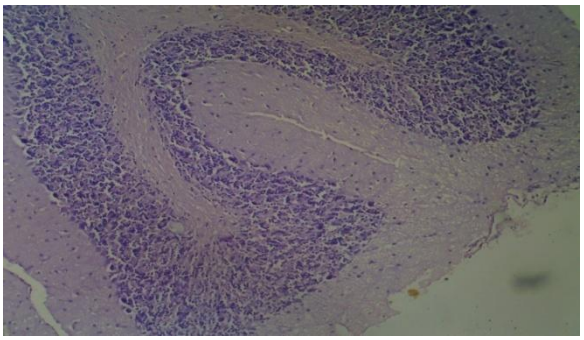
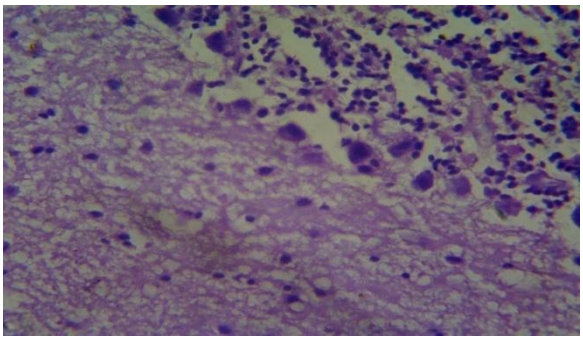
ONLY MPTP (20 mg/kg)

From the results the Mice brain cerebrum shows focal neuronal injury. Also show mononuclear inflammatory infiltration in the brain parenchyma with mild alter in the morphology and number of neurons. The molecular purkinjiec layer shows normal morphology.

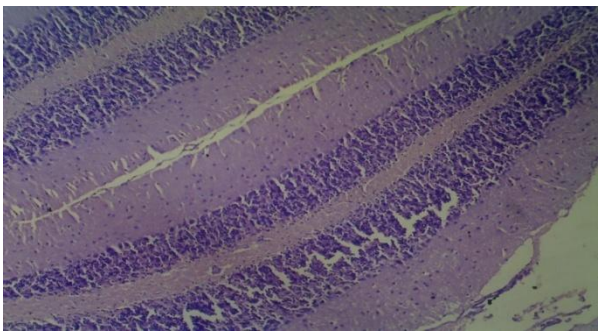
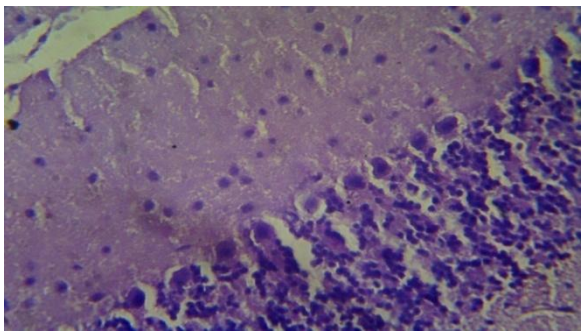
10X MPTP+ LEVODOPA/BENZERAZIDE 40X

From the results Mice brain with cerebellum and cerebral cortex shows degeneration. Hippocampus shows atrophy. Para hippocampal cortex shows degeneration and mild gliosis. Section from cerebrum shows sclerosis and degeneration. Thalamus, amygdale and substantia nigra shows no significant pathology.

10X MPTP+ AEBP (200mg/kg) 40X

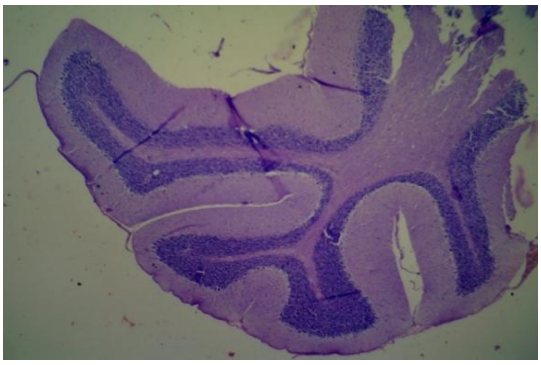
From the results the Mice brain with cerebellum and cerebral cortex shows degeneration. Hippocampus shows atrophy. Para hippocampal cortex shows degeneration and mild gliosis. Section from cerebrum shows sclerosis and degeneration. Thalamus, amygdale and substantia nigra shows no significant pathology.

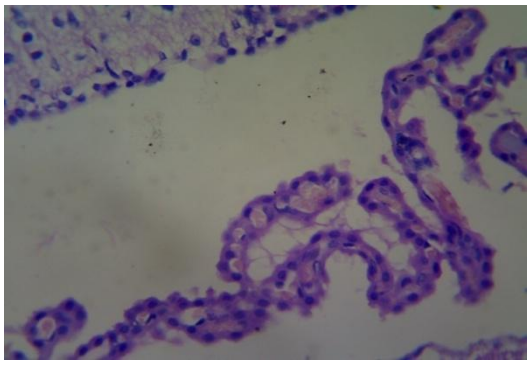



10X MPTP+ AEBP (400mg/kg) 40X

From the results the Mice brain with cerebellum shows normal molecular purkinjiec cell layers. Hippocampus shows gliosis and degeneration. Section from cerebral cortex shows severe gliosis and degeneration. Corpus striatum, thalamus and amygdala shows no significant pathology. Substantia nigra and choroid plexus are no significant pathology.

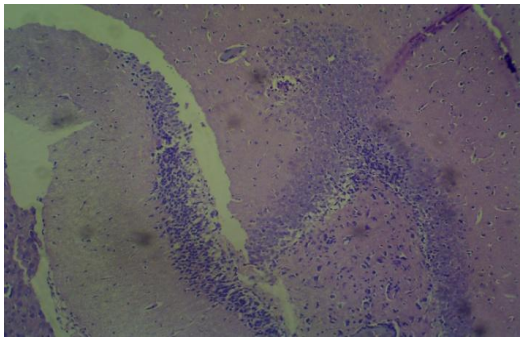
HISTOPATHOLOGICAL EVALUATION ROTENONE INDUCED PARKINSONISM

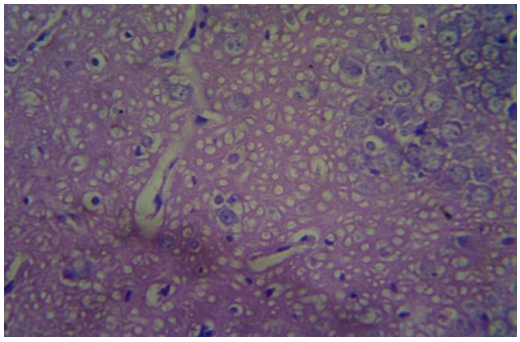




10x ROTENONE + AEBP (200mg/kg) 40x

From the results the Rat brain with cerebrum shows mild gliosis. Hippocampus shows normal granular and pyramidal cell layer. There is no disorganization/ degeneration are seen. Section from cerebellum shows no significant pathology (normal molecular, granular and purkinjiec layers). Parahippocampal cortex shows mild gliosis. Thalamus and amygdala shows no significant pathology.





10x ROTENONE + AEBP (200mg/kg) 40x

From the results the Rat brain with cerebellum shows normal normal molecular purkinjiec cell layers. Hippocampus shows mild gliosis and vacuolar degeneration. Section from cerebrum shows mild gliosis. Corpus striatum also shows mild gliosis. Thalamus and amygdala shows no significant pathology. Mild frontal cerebral cortex gliosis are seen. Substantia nigra and choroid plexus are no significant pathology.

5. DISCUSSION

This study aims to investigate the Neuroprotective effect of *Barleria prionitis* on MPTP and Rotenone intoxicated animal models of PD by analyzing behavior patterns, brain antioxidant, brain neurotransmitters and Histopathological studies. PD is a commonly occurring neurodegenerative disorder that produces muscular rigidity, bradykinesia, tremor in resting limbs and loss of postural balance. The basic neuropathology of PD involves the selective degeneration of dopaminergic cells in specific brain regions like the striatum; when degeneration in these neurons reaches a threshold reduction of 80% dopamine, the motor symptoms of PD emerge. The cerebral cortex, which is abundant with neurons and easily accessible, demonstrates its importance in modelling PD.

Barleria prionitis has a long history in herbal medicine in various countries. The leaves and bark of *Barleria prionitis* have long been used as a natural medicine in tropics. The bark and leaf extract of *Barleria prionitis* is well known for its different types of pharmacological properties such as Stomach disorder, Urinary infections, fever, Tooth ache, Diuretic, Jaundice, Asthma, Arthritis, Inflammation, Migraine, Dropsy, Leprosy. The major active constituents are Iridoids, Barlerin, Acetyl barlerin, β-sitosterol, Prioniside, Barlerinoside, verbascoside, 7-methoxy diderroside, Anthroquinones, Flavonoids. Since *Barleria prionitis* have not been studied for its anti-parkinson activity, the present study was aimed to evaluate the anti-parkinson activity potential of aqueous extracts of *Barleria prionitis* in MPTP and rotenone induced swiss albino mice and SD rats respectively.

MPTP was adapted as a PD model in rodents and primates. These studies showed that this compound acts as a lipophilic protoxin that crosses the BBB and is converted to the toxic metabolite 1-methyl-4-phenylpyridinium (MPP+) by astrocytes and serotonergic neurons[90] by monoamine oxidase-B[91]. MPP+ is then released into the extracellular space and accumulated by the DA transporter (DAT) into DA neurons, causing a bilateral degeneration of the nigrostriatal tract[92]. MPP+ produces neurodegeneration through the blockade of electron transport chain enzyme complexes I, III, and IV[93]. Additional factors modulate MPTP toxicity including iron,

expression of the vesicular monoamine transporter, reactive oxygen species (ROS), and apoptosis[94,95].

Exposure to the pesticide rotenone is linked to an increased risk of PD in epidemiological studies[96,97,98], and have been adapted for PD models. Rotenone is highly lipophilic and crosses the BBB to diffuse into neurons where it inhibits complex I of the mitochondrial respiratory chain and causes neurodegeneration of SN neurons; however, reports of its selectivity for neurotoxicity are variable[100].

Effect of *Barleria prionitis on* MPTP and Rotenone-induced PD:

Effect on behavioral parameters

Actophotometer is used for screening the locomotor and anti-anxiety activity in rodents, while the rotarod for muscle relaxant activity. Locomotor activity indicates attentiveness and the decline indicates sedative action. The GABA receptor compound is concerned in sedation, muscle relaxant and anxiety in CNS. Various neurological and mental disorders such as epilepsy, depression, Parkinson syndrome, Alzheimer's disease are involved with this receptor.

The increase in locomotor activity OF extract of *Barleria prioitis* has shown stimulant effect in actophotometer. This provoked to evaluate it further, using paradigms of depression models. From the results the Actophotometer readings (locomotor activity) of animals of vehicle-treated control group (Group I) was found to be 386.33±1.56 and 396±1.15 counts/5 min for all 7 days of treatment.

MPTP and Rotenone treatment to animals of Group II showed a significant reduction in locomotor activity every week. The actophotometer readings decreased to 186±2.30 and 195.33±8.04 counts/ 5 min. on the 7th day, went down to 145.33±0.23 and 157.67±1.42 counts/5 min. Thus, there was a significant decline in the locomotor activity of rotenone treated control animals (Group III) when compared to vehicle-treated control group (Group I).

Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced a significant increase in locomotion (246.33±0.77, 215.67±0.56, 236.67±1.85s and 258.33±2.42, 224.33±2.90, 246.33±2.18 counts/ 5 min.) on the 7th day of

treatment; as compared to the MPTP and Rotenone treated control (Group II) animals on the respective days.

Rota rod test a standard animal model used to evaluate peripheral neuromuscular blockade and the motor coordination, a deficit in motor coordination would very likely affect performance in the behavioral tests. Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation.From the results the Rota rod readings (fall off time) of Vehicle-treated Control group (Group I) animals from the rota rod was found to be 178.67±0.96 and 130±1s. MPTP and Rotenone treatment to animals of Group II showed a significant reduction in the muscle grip or strength every week. The rotarod fall off times decreased to 74.667±1.33 and 69.667±1.20s. counts/5 min. on the 7th day, went down to 61.333±0.18 and 43.333±9.98s counts/5min. Thus, there was a significant decrease ($p<0.001$) in the muscle activity of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I).

Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly increased the muscle activity (120.67±0.59, 94.667±0.72, 111.67±0.14s and 118.67±6.75, 95±7.48, 111.33±3.52) on the 7th day of treatment; as compared to the MPTP and Rotenone -treated control (Group II) animals on the respective days.

Due to their immobility time, muscular coordination skill memory, motor impairment, retention times were also decreased. From the results the Tail suspension test readings (total immobility time) of Vehicle-treated Control group (Group I) animals was found to be 53±1.52 and 55±4.46s. MPTP and Rotenone treatment to animals of Group II showed a significant increase in the immobility time every week. The tail suspension immobility times increased to 129.67±3.33 and 132.67±1.76s. sec/6 min. on the 7th day, went up to 131±2.47 and 136±2.32s sec/6min. Thus, there was a significant increase ($p<0.001$) in the immobility time of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I).

Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly decreased the muscle activity (87.667±0.18, 121.33±4.93,

96±10.83 and 92±2.30, 124±1.97, 102.67±2.78 s) on the 7th day of treatment; as compared to the MPTP and Rotenone -treated control (Group II) animals on the respective days.

Forced swimming test readings (total immobility time) of Vehicle-treated Control group (Group I) animals was found to be 48.667±1.33 and 53±1.94s. MPTP and Rotenone treatment to animals of Group II showed a significant increase in the immobility time every week. The immobility times increased to 125±0.73 and 128.67±0.85 s. sec/6 min. on the 7th day, went up to 131.33±2.37 and 132.67±1.45s sec/6min. Thus, there was a significant increase ($p<0.001$) in the immobility time of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly decreased the muscle activity (85.333±1.78, 118.67±2.27, 93.333±0.15s and 90±1, 121.33±3.09, 99.667±1.22) on the 7th day of treatment; as compared to the MPTP and Rotenone -treated control (Group II) animals on the respective days.

The hole board test is helpful for modeling anxiety in animals, in this test an anxiolytic-like state may be reflected by an increase in head –dipping behaviors. From the results the Hole board test readings (No.of head dippings) of Vehicle-treated Control group (Group I) animals was found to be 33.667±2.46 and 29±2.42. MPTP and Rotenone treatment to animals of Group II showed a significant decrease in the head dippings every week. The hole board test head dipping decreased to 22±3.68 and 21.667±1.76 sec/5 min. on the 7th day, went up to 18.333±4.82s and 14±2.51 sec/5min. Thus, there was a significant decrease ($p<0.001$) in the head dipping of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I).Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly increased the head dipping (31.333±0.88, 21.333±0.91, 27.333±1.20 and 27.333±0.66, 18.667±3.01, 23.333±0.31) on the 7th day of treatment; as compared to the MPTP and Rotenone -treated control (Group II) animals on the respective days.

Effect on Brain antioxidant levels

The biotransformation of MPTP into MPP+, which is catalyzed by the mitochondrial enzyme monoamine oxidase B, represents the major route for MPTP-mediated neurotoxicity[101]. The conversion of MPTP to MPP+ has been suggested to induce the formation

of ROS. This notion is supported by previous studies which showed increased superoxide (O2·−) and hydroxyl radical (·OH) levels during the biotransformation of MPTP[102]. While the damage induced by O2·− is limited, it can react with nitric oxide (NO) to form peroxynitrite (ONOOO−) which readily forms the more reactive ·OH radical. Other studies have shown that MPTP induces toxicity through ATP depletion and mitochondrial dysfunction. Moreover, that ATP depletion plays a major role in MPTP induced neuronal cell death [103]. However, it is likely that MDA can form complexes with other biological components such as protein, lipids, and nucleic acids which can contribute to an underestimation of endogenous lipid peroxidation[104]. On the contrary to our lipid peroxidation data, we also show that MPTP can lead to distinct alterations in endogenous antioxidant defense mechanisms. MPTP treatment has been previously shown to significantly increase Mn-SOD and CuZn-SOD activities in the striatum of C57BL/6 mice, which is suggestive of acute oxidative stress insult[105]. SOD is upregulated in cells when O2 ·− is produced in excessive levels[106]. This observation suggests that SOD may play a role in the toxicity observed following acute treatment of MPTP, although ROS formation may not play a major role in MPTP-induced toxicity. CAT is an enzyme that is involved in the detoxification of ROS and the elimination of hydrogen peroxide (H2O2) in particular[107]. The increase in both intracellular SOD and CAT activities may therefore represent an adaptive response due to the leakage of free radicals during impaired mitochondrial respiration. Treatment with MPTP also leads to reduced activity of GPx and decreased levels of the essential pyridine nucleotide NAD+, ATP, and GSH in primary human neurons after a 24-hour exposure. The maintenance of GPx activity appears crucial for the maintenance of cell viability during oxidative insult [108-111]. Moreover, previous studies have shown that MPP+, the metabolite of MPTP induces GSH depletion without increasing the levels of oxidized glutathione disulfide (GSSG) [112]. Reduced GSH levels may occur due to that MPP+ induced decline in intracellular NAD+ and ATP stores which are necessary for GSH anabolism, release, and taken together, our data suggests that MPTP exposure can limit the endogenous antioxidant defense, subsequently increasing the vulnerability of neuronal cells to additional oxidative stress. An imbalance in the function of endogenous antioxidant defense mechanisms can lead to the accumulation of free radicals and

ROS and increased susceptibility to oxidative stress, which contributes to the pathogenesis of PD.

Effect of *Barleria prionitis* on LPO level in brain:

The LPO level in Control group (Group I) animals was found to be 6.1547±0.05 and 3.7267±0.01nmoles of MDA/mg protein. Administration of MPTP and Rotenone (Group II) resulted in a significant increase in LPO level in brain of animals as compared to Control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) showed a significant reduction in the level of LPO in brain as compared to MPTP and Rotenone treated animals (Group III).

Effect *Barleria prionitis* on GSH level of brain:

The level of GSH in the brain homogenate of Vehicle treated Control group (Group I) animals was found to be 6.4933±0.001and 5.0733±0.04µg/mg protein. MPTP and Rotenone treatment resulted in a significant decrease in GSH levels in brain of Group II animals as compared to control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) significantly increased GSH levels in the brain as compared to MPTP and Rotenone treated animals (Group III) thus preventing the reduction in GSH induced by rotenone.

Effect on Brain Neurotransmitters levels

MPTP selectively damages the dopaminergic nigrostriatal system, resulting in the loss of dopaminergic neurons in the substantia nigra and a depletion of dopamine in the striatum[113]. The loss of dopaminergic neurons is associated with an onset of motor symptoms, and there is a direct relationship between extent of dopamine loss and motor dysfunction [114]. The striatum is considered to be the region responsible for head and forelimb motor control. Many studies have revealed impaired behavioural responses within a short span of MPTP lessoned animals[115]. The neurotransmitter, DA plays a key role in body movement and motor control. A well known fact is that there is a reduced level of Dopamine occurring in dopaminergic neuronal damaged brain [116]. Dopamine neurotransmitter was more affected in Parkinson's disease [117,118]where as other brain amines like norepinephrine, epinephrine and serotonin were much less affected than

dopamine in MPTP treated group[119], because main targeted neurotransmitter is dopamine in Parkinson's disease. These findings are near to previously reported study[120].

Estimation of dopamine assay

The MPTP and Rotenone treated group of animals had decreased dopamine levels when compared to the control group. Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) the dopamine activity showed significance increased in ($p<0.001$, $p<0.01$) on comparsion with the MPTP and Rotenone treated group shown as Table 21 and 14 . The data showed that the *Barleria prionitis* has a very protective role of dopamine in the receptor binding density of mice with *Barleria prionitis*.

Estimation Serotonin

The MPTP and Rotenone treated group of animals had decreased **Serotonin** levels when compared to the control group The serotonin activity was observed in the MPTP and Rotenone group mice when compared to the control group ($p<0.01$). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) groups showed significance increase in **Serotonin** levels ($p<0.001$, $p<0.01$) when compared with the MPTP and Rotenone group shown in Table 21 and 14 .

Estimation of GABA

Table 21 and 14. depicts that the effect of Gamma –amino butyric acid levels decrease in MPTP and Rotenone treated group of animals when compared to the control group group ($p<0.001$) .Whereas Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) treated mice was observed significance increase decreased ($p<0.001$, $p<0.01$) as compared with MPTP group.

6. CONCLUSION

From the present study, it can be considered that the aqueous extract of *Barleria prionitis* exhibited significant anti-parkinsonism activity in MPTP and rotenone model in mouse and rats respectively. The probable mode of action of this plant decreased lipid peroxidation due to the presence of flavonoids, polyphenols and glycosides. All the Parameters of extract treated group animals have shown better results when compared with MPTP and Rotenone-induced group and the standard L-dopa treated group. These findings provide a preliminary evidence for its potential as anti-parkinsonian medication, including Parkinson's disease prevention and improvement of symptoms.

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