Evaluation of Neuroprotective Role of Drugs That Modify Renin Angiotensin System on Histoanatomical Structures of Brain in Animal Models of Parkinson's Disease

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By

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LIST OF ABBREVIATIONS USED

ACh	Acetyl choline
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitor
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
AT_1 receptor	Angiotensin 1 Receptor
Bcl-2	B-cell lymphoma 2
BW	Body Weight
CAT	Catalase
COMT	Catechol-O-Methyl Transferase
COX	Cyclooxygenase
CPCSEA	Committee for the Purpose of Control and Supervision of Experiment on Animals
DA	Dopamine
DAB	3,3'-Diaminobenzidine
DBS	Deep brain stimulation
DPX	Dibutylphthalate Polystyrene Xylene
EDTA	Ethylenediaminetetraacetic acid
fig.	Figure
GABA	Gama-Aminobutyric acid
gm	Gram
GDNF	Glial cell line-derived neurotrophic factors
GSH	Reduced Glutathione

Hcl	Hydrochloric Acid
HEF	Hepatocyte Growth Factor
H & E	Haematoxylin and Eosin
hr	Hour
Hz	Hertz
5-HT	5-Hydroxytryptamine [Serotonin]
i.p	Intraperitoneal
IAEC	Institutional Animal Ethical Committee
kg	Kilogram
LPO	Lipid peroxidase
LB	Lewy Bodies
M	Molarity
mA	Milliampere
mg	Milligram
mg/dl	Milligram/Decilitre
mg/kg	Milligram Per Kilogram
mg/ml	Milligram/ Millilitre
mL	Millilitre
min	Minute
mM	Millimole
mm	Millimetre
µg	Microgram
MPO	Myeloperoxidase
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine

NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PD	Parkinson's disease
RAS	Renin Angiotensin System
s.c	Subcutaneous
Sec.	Seconds
SEM	Standard error mean
SNc	Substantia Nigra pars compacta
SNr	Substantia Nigra pars reticulata
SOD	Superoxide Dismutase
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organisation

ABSTRACT

Background and objectives

Current parkinsonian treatments do not address the disease's aetiology or development. Routine drugs rarely affect issues of neuronal protection and endurance in dopaminergic neurons. With deeper understanding of brain renin-angiotensin system, many angiotensin converting enzyme inhibitors and angiotensin receptor blockers are evaluated for the management of parkinsonism.

The main goal of this study was to evaluate and compare the anti-disease parkinson's properties of captopril, perindopril, losartan, and the standard anti-parkinson's disease drugs (levodopa) in rotenone, MPTP, and paraquat induced models in wistar albino rats and swiss albino mice with the standard anti-disease parkinson's disease drugs (levodopa).

The other objective was to evaluate and compare the neuroprotective role of captopril, perindopril and losartan on histoanatomical structures of brain in rotenone, MPTP and paraquat induced parkinson’s disease animal models in wistar albino rats and swiss albino mice.

Methodology:

Healthy adult wistar albino rats of either sex weighing 180-250gm were selected and divided into six groups, each containing six animals in rotenone model. Similarly, healthy adult swiss albino mice of either sex weighing 20-30gm of six groups, each containing six animals were selected for MPTP and paraquat models separately. All the rodents were obtained from the animal house; Institutional Animal Ethical Committee approved before the start of the study. Effects of captopril (20 mg/kg), perindopril (5 mg/kg) and losartan (90 mg/kg) were evaluated in rotenone,

MPTP and paraquat models. Neurobehavioral effects were noted through spontaneous locomotor activity, rotarod test, hole board test, forced swim test, tail suspension test and elevated plus maze test. After documenting the neurobehavioral parameters the rodents were anaesthetized and sacrificed, the brain tissue was extracted by dissection method. Oxidative stress markers, neurotransmitters and inflammatory marker were evaluated in one hemisection. Other hemisection was H & E stained for analysing histoanatomical changes, and Bcl-2 immunohistochemistry study was done to evaluate the anti-apoptotic effects of these drugs.

Results

Perindopril and losartan partially improved motor functions in rotenone, MPTP and paraquat models. All the drugs had shown anti-depressant action in all the three models. Perindopril and losartan had shown anti-anxiety action. Captopril, perindopril and losartan had exhibited neuroprotective role as evidenced by the decreased glutamate levels in all the three models. Captopril, perindopril and losartan had documented the neuroprotective role as evidenced by improved oxidative stress marker levels in all the three models. Captopril, perindopril and losartan had proved greater neuroprotective role as evidenced by the increased serotonin, dopamine and acetylcholine levels in rotenone and MPTP models.

Captopril, perindopril and losartan had not resulted in any significant histoanatomical changes in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus sections as H&E sections, and shown near normal histoanatomy. Captopril and perindopril had shown significant anti-apoptotic property as evidenced through Bcl-2 immunohistoreactivity in rotenone and paraquat model respectively.

Conclusion

Overall, captopril, perindopril and losartan had significantly improved the non-motor behavioural aspects of PD. All the three drugs significantly decrease the oxidative stress levels inferring that, they are neuroprotective in all the three models.

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INTRODUCTION

INTRODUCTION

Context of the study

Parkinson's disease [PD] is a progressive neurological condition, second among all the chronic neurodegenerative disorders, trailing only alzheimer's disease [AD][1]. Around 50 lakh people worldwide suffer from this disease, which has cardinal features such as rigidity, resting tremors, bradykinesia, and gait disturbances, as well as other symptoms such as difficulty in speaking, sensory alterations, sleep disturbances, autonomic disturbances, motor disorders, postural instability and dementia[1].

Chronic degeneration of neurons carrying dopaminergic axons in the substantia nigra pars compacta [SNc] with proteinaceous inclusions in the cytoplasm known as Lewy bodies is a pathologic feature of PD[1-3]. Chronic neuronal degeneration of cholinergic neuronal fibres of the nucleus basalis of Meynert [NBM], serotonergic fibres of nuclei of the brainstem, adrenergic fibres of the locus ceruleus [LC], neuronal fibres in cerebral hemispheres, and the olfactory system are also other important histoanatomical structural changes in PD[1].

Currently available drugs[1-3] for PD include Dopamine precursor [levodopa] in combination with peripheral decarboxylase inhibitors [carbidopa, benserazide], Monoamine Oxidase-B [MAO-B] inhibitor [selegiline], Catechol-O-Methyl Transferase [COMT] inhibitors [tolcapone, entacapone], dopaminergic agonists [bromocriptine, pramipexole, ropinirole] and central anticholinergics [trihexyphenidyl, biperiden]. Anti-parkinson’s disease drugs that are currently available only address the symptoms of the condition without stopping the degeneration of dopaminergic neurons in the brain4.

The Renin-Angiotensin-Aldosterone [RAS][5] system regulates the body water balance, blood pressure, sympathetic pathway activation, and the vasopressin synthesis & release. The RAS system in the brain is unique from the peripheral RAS in the body, having three subtypes of angiotensin II receptors: AT1, AT2 [both G-protein coupled receptors], and AT4. Area postrema, inferior olivary nucleus, anterior pituitary, anterior ventral third ventricle region, lateral geniculate body, ventral tegmental area, the nucleus of the solitary tract, subfornical organ, median eminence, paraventricular, preoptic and supraoptic nuclei of the hypothalamus are having the AT1 subtype of angiotensin II receptors. In the inferior olivary nucleus, amygdala, locus ceruleus, hypoglossal nucleus, thalamus, medial geniculate body, habenula, corpus striatum, ventral tegmental area and inferior colliculus, the AT2 subtype of angiotensin II receptors are richly found.

It has been established[6] that the central RAS contributes significantly to the pathogenesis of PD. Angiotensin II works as a proinflammatory mediator in the brain, producing reactive oxygen species[ROS] and activating the NADPH-dependent oxidase complex, resulting in oxidative stress resulting in the dopaminergic neuronal fibre loss[6]. According to a preclinical investigation in rats[7], the angiotensin-converting enzyme [ACE] controls the turnover of dopamine content in the basal ganglia; according to a preclinical investigation in rats[7], increased brain RAS expression has been linked to the vulnerability of dopaminergic fibres carrying neurons[8]. According to the studies, brain angiotensin II induces oxidative stress, inflammation amplification, microglial cell activation, and all finally culminate to cause dopaminergic neuron death[9].

Several animal experiments had shown that the drugs modifying brain RAS have a significant role in the treatment of PD symptomatology. In PD animal models

and clinical research, few angiotensin-converting enzyme inhibitors [ACEI][10-15] and angiotensin-receptor blockers [ARBs][16] have shown a promising role in the management of PD.

Despite the fact that there are many solid research publications on PD, further studies focusing on the varied Indian population, particularly on therapeutic issues, are considered essential. In our setup, results from western literature may not be completely extrapolated. This opened the door for us to gain insight into PD and explore newer therapeutic approach.

Hence, evaluation of the neuroprotective role on histoanatomical changes in brain structures and anti-PD properties of ACEI and ARBs in various experimental animal models is warranted for the further better pharmacotherapy for PD.

Despite the fact that many research evaluating various drugs and molecules in a many animal models are conducted around the world, there are only few attempts to evaluate a group of drugs at a time and build a corpus of comparative data. Therefore, this study was designed to evaluate three drugs at a time in three rodent models. This was committed in establishing an extensive information on the effects of these drugs in PD in rodent models. Apart from that, most of the previous studies in this field are confined to a single outcome, such as behavioural analysis, estimation of neurotransmitter levels, or oxidative stress assessment. But the attempt had been made to incorporate all of these outcomes in this present single study for all the drugs and in all the three rodent models. Neuro-behavioral analysis, oxidative stress enzyme assessment, assay of neurotransmitters and inflammatory marker levels, histoarchitectural evaluation, and immunohistochemistry study were among the outcomes in the study.

Justification of the study

Anti-parkinson's disease drugs that are currently available control only the symptoms, and have been associated with major side effects when used for a long term. Behavioural defects [severe depression, mania, mental confusion, psychosis, etc.], abnormal movements [choreoathetoid limb movements, grimacing, facial tics, etc.], and fluctuation in motor function are the major long-term toxicities of current anti-parkinson's disease drugs.[1-3]

Increased expression of ACE results in an increased synthesis of angiotensin II, which acts through central AT1 subtype receptors to produce an environment of oxidative stress leading to the degeneration of dopaminergic neuronal fibres that is attributed to the pathogenesis of PD.[6-9]

Several previous studies had shown that some ACE inhibitors and ARBs are effective in the treatment of PD.[10-16] ACE inhibitors [enalapril, lisinopril, captopril, fosinopril, ramipril, perindopril] and ARBs [candesartan, valsartan, telmisartan, losartan, etc.][1-3] are currently used to treat a range of cardiovascular conditions, including hypertension, and have shown extremely safe and tolerable over the long term use.[1-3]

There are only a few research in the literature that have evaluated the anti-parkinson disease properties of ACE inhibitors and ARBs, and all those studies have only been done for a few ACE inhibitors and in a few animal models. In the literature, there is no single large study that has evaluated and compared the neuroprotective effects of ACE inhibitors and ARBs on histoanatomical changes in the brain in experimentally induced PD in various animal models.

There is a need to understand still better and in detail about the treatment for PD at the level of brain tissue with respect to histoanatomical changes. It is also

necessary to have various pharmacological interventions for PD that will not only reduce the occurrence of symptoms but also have a neuroprotective effect on the brain's histoanatomical changes. As a result, extensive research and experimentation on various animal models are required to understand the neuroprotective effects of drugs that affect RAS on histoanatomical changes in the brain in order to develop more effective treatments for PD in the future for the betterment of mankind.

HYPOTHESIS

HYPOTHESIS

Drugs that modify the renin-angiotensin system (captopril, perindopril, and losartan) exhibit significant anti-parkinson's disease properties as well as a significant neuroprotective effect on histoanatomical structures of brain in rotenone, MPTP and paraquat induced experimental models in wistar albino rats and swiss albino mice.

AIMS & OBJECTIVES

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Aim of the study:

To evaluate the neuroprotective role on histoanatomical changes in the structure of brain and anti-parkinsons disease properties of ACEIs and ARBs in the experimental animal models [Rotenone, MPTP and Paraquat]

Objectives:

i. To evaluate and compare the anti-parkinson's disease properties of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) in **Rotenone** induced experimental models in wistar albino rats.

ii. To evaluate and compare the anti-parkinson's disease properties of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) in **MPTP** induced experimental models in swiss albino mice.

iii. To evaluate and compare the anti-parkinson's disease properties of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) in **Paraquat** induced experimental models in swiss albino mice.

iv. To evaluate and compare the neuroptotective role of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) by assaying the levels of antioxidant enzymes and neurotrtransmitters, inflammatory marker, histopathological and

immunohistochemistry examination of brain in **Rotenone** induced experimental models in wistar albino rats.

v. To evaluate and compare the neuroptotective role of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) by assaying the levels of antioxidant enzymes and neurotrtransmitters, inflammatory marker, histopathological and immunohistochemistry examination of brain in **MPTP** induced experimental models in swiss albino mice.

vi. To evaluate and compare the neuroptotective role of drugs modifying renin angiotensin system (Captopril, Perindopril and Losartan) with the standard drug (Levodopa) by assaying the levels of antioxidant enzymes and neurotrtransmitters, inflammatory marker, histopathological and immunohistochemistry of brain in **Paraquat** induced experimental models in swiss albino mice.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Parkinson’s disease

Parkinson's disease (PD) is a progressive neurological disease. PD is the second most common neurodegenerative disease after alzheimer's disease, with more than 17 million people affected. Tremors at rest, rigidity, bradykinesia (slowing of movement), and postural instability are the four cardinal motor symptoms. Intracytoplasmic inclusions from the protein aggregates called Lewy Bodies (LBs), and a decrease in pigmented dopamine-containing neurons in the substantia nigra pars compacta of the midbrain are the pathological indicators of parkinson's disease (PD). The loss of 50-70 percent of dopaminergic neurons in the substantia nigra is a hallmark of PD. Thus, both the cause and the mechanism of PD are currently unclear.[17 - 20]

There is no evident genetic relationship in about 95% of PD cases, which is referred to as "sporadic PD," but the disease is inherited in the remaining cases. Current data suggests that both environmental and genetic factors play a role in the progression of PD; researchers developed animal models of PD on this basis of pathogenesis. These models are based on the systemic or local delivery of neurotoxins capable of reproducing clinical and behavioural changes similar to those seen in PD in the mammals. Treatment with levodopa is still the gold standard for PD therapy.[19] Unfortunately, long-term usage of L-dopa causes dyskinesias (involuntary movements).[21] Current therapeutic approaches are only symptomatic; none of them slow down/stop the loss of dopaminergic neurons.

Thus, developing animal models is critical for gaining a better knowledge of the pathophysiology and progression of PD, as well as for the therapeutic discovery.[22,23]

Aetiology and pathogenesis

The causes of neuron degeneration in PD are yet unknown. Heredity appears to play a limited role in most of the cases. The oxidative stress theory is one of the more well-known theories related to the causes of PD.[24] In the basal ganglia, metabolic oxidation of dopamine generates highly reactive free radicals that are toxic to dopaminergic neurons and lead to their degeneration. Free radicals are the molecules that lack an electron in their outer orbits and are capable of extracting electrons from other molecules, resulting in the cell damage.

The corpus striatum (caudate and putamen), substantia nigra, globus pallidus, and subthalamus are among the interconnected subcortical nuclei that make up the basal ganglia. The basal ganglia receive input from the cerebral cortex, process it, and then deliver feedback to the brain's motor cortex region in a manner that enables healthy persons to coordinate their body movements smoothly. Even simple actions like walking involve a comprehensive sequence of motor acts involving the continual connection between the cortex and the basal ganglia for the smooth execution. Neuronal deterioration disrupts this connection in the people with PD.

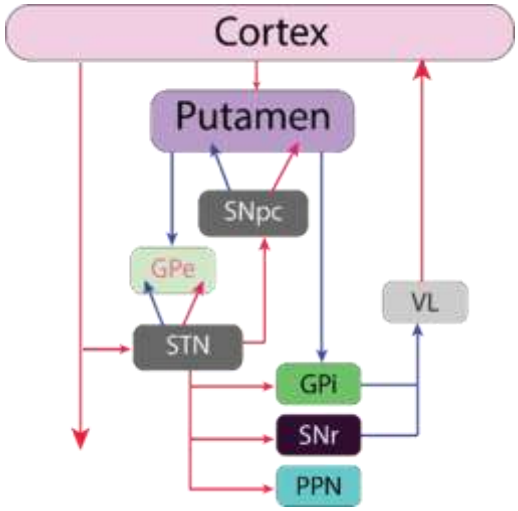

Figure 1: Normal connections of basal ganglia, inhibitory connections are shown as blue arrows and excitatory connections as red arrows

[GPe - External segment of the globus pallidus; GPi - Internal segment of the globus pallidus; SNr - Substantia nigra, pars reticulata; SNc - substantia nigra pars compacta; STN - Subthalamic nucleus; VL - Ventrolateral thalamus; PPN - Pedunculopontine nucleus]

The corpus striatum takes information from the entire cerebral cortex and the substantia nigra, and sends projections to the thalamus via the globus pallidus, substantia nigra, and subthalamus via direct and indirect pathways. D1 dopamine receptors in the corpus striatum stimulate the direct pathway, while D2 receptors inhibit the indirect pathway. Degeneration of dopaminergic neurons results in decreased direct pathway activity and increased indirect pathway activity in PD. As a result of these alterations, the thalamic input to the motor cortex is diminished, and the patient develops stiffness and bradykinesia[25]

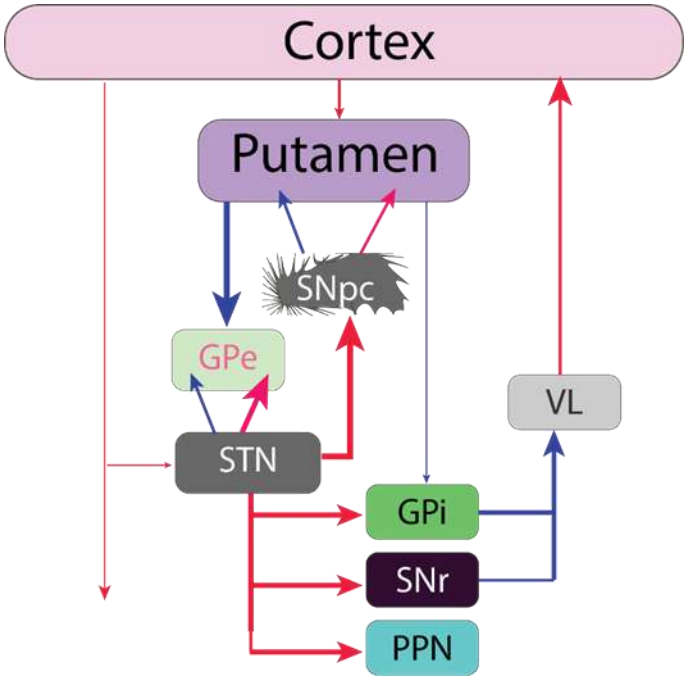

Figure 2: Connections of basal ganglia in parkinson's disease, inhibitory connections are shown as blue arrows and excitatory connections as red arrows.

[GPe - External segment of the globus pallidus; GPi - Internal segment of the globus pallidus; SNr - Substantia nigra, pars reticulata; SNc - substantia nigra pars compacta; STN - Subthalamic nucleus; VL - Ventrolateral thalamus; PPN - Pedunculopontine nucleus.

Signs and symptoms:

Motor Manifestations[25,26]

- Tremor: When the limbs are at rest, the tremor is at its highest, and it decreases with voluntary movement.
- Rigidity: the rigidity or resistance of limbs to passive movement.
- Bradykinesia is characterised by sluggishness and a lack of movement.
- Postural instability: Postural reflexes fail, resulting in a loss of balance and a fall.

The following are the some more motor symptoms:

- Gait freezing [Motor block]: A sudden inability to take a stride forward while walking is referred to as gait freezing. It's a brief occurrence that lasts seconds or minutes before disappearing.
- Dystonia: an abnormal, long-lasting, painful twisting of muscle contraction, which frequently affects the foot and ankle (mainly toe flexion and foot inversion).
- Hypophonia [soft speech] is characterized by soft, hoarse and monotonous speech.
- Masked faces (a mask-like face, also known as hypomania), with occasional blinking
- Micrographia (small, cramped handwriting)
- Impaired fine motor dexterity and coordination

Levodopa resistant motor and non-motor symptoms develop as non-dopaminergic brain regions get involved as the disease progresses.

Cognitive and psychiatric manifestations[19,27–28]

- Dementia: Slowing of thought that progresses to difficulties with abstract thought, memory and behavioural regulations.
- Depression: About 47% of people with PD are depressed.
- Impaired short-term memory.
- Hallucinations, delusions, anger, apathy and anxiety.

Risk factors of Parkinson's disease

Many environmental factors, including acute and chronic pesticide, herbicide, and insecticide exposure, have been observed in human epidemiological research. Numerous inherited variables have also been implicated in PD progression.

Table 1: Tabulation of environmental and genetic factors found to increase the risk of PD

Environmental factors	Specific agents	Mechanism of PD
Herbicides and pesticides	Paraquat (bipyridyl derivatives) Maneb Rotenone	In a rodent model, it exacerbates dopaminergic degeneration. In humans, the incidence of PD is higher.[29-32] It is a powerful complex inhibitor.[32]
Cigarette	People with a history of smoking have a 60% lower risk of developing PD than those who have never smoked.[33]	Nicotine enhances dopamine release, whereas cigarette smoking reduces monoamine oxidase function.
Caffeine	When compared to non-coffee users, coffee consumers may have a 30% lower risk of PD.[34]	It inhibits the production of free radicals and protects dopaminergic cells from death.
Dual hit theory	Pathogens that cause α-synuclein aggregation can reach the neurological system through the nasal and intestinal epithelium[35,36]	Degeneration of neurons is the extension of peripheral disease process starting at enteric nervous system
Genetic factors	Nature of components	Mechanism of PD
Synuclein	Ala53 →Thr (A53T) Ala3→Pro (A30P)	Result in dominantly inherited PD.[37,38]
Parkin	Component of ubiquitin-proteasome system	Heterozygote mutations in parkin lead to PD. [39,40]
PINK 1 gene	The mitochondrial targeting domain and the kinase domain	The G309D mutation results in a loss of neuroprotective properties[41,42]

Classification of drugs used in the treatment of Parkinsonism

I. Drugs that impact the dopaminergic system in the brain[43]

- Levodopa, which is a dopamine precursor.
- Dopamine metabolism inhibitors include:

Tolcapone and Entacapone are COMT inhibitors.

Selegiline and Rasagiline are two MAO-B inhibitors.

- Amantadine, a dopamine agonist.
- Agonists of the dopaminergic system:

Some of the ergot derivatives available are bromocriptine, pramipexole, and ropinirole.

Lysuride is an example of a non-ergot derivative.

II. Drugs that impact the cholinergic system in the brain[44] are classified into two categories.

- Procyclidine and benzhexol are central anticholinergics.
- Promethazine and orphenadrine are antihistaminics

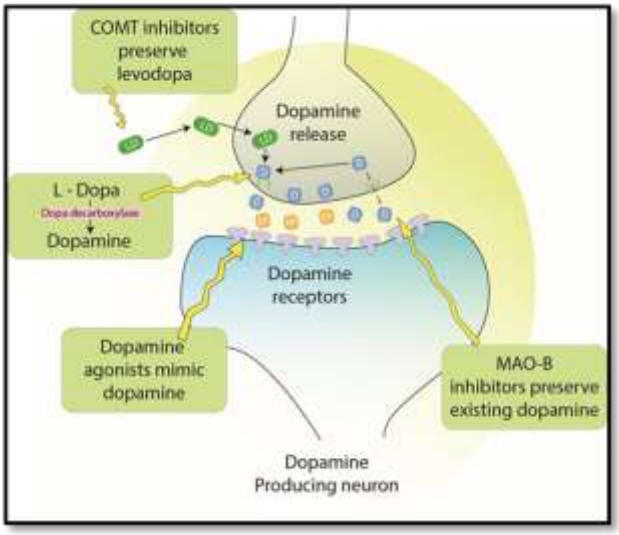

Figure 3: Site of action of medications for the treatment of motor symptoms

Limitation of current therapies

Table 2: Tabulation of currently used drugs in parkinsonism therapy with their major limitations

Drug	Comments	Limitations
Levodopa[21]	Rapidly decarboxylated when administered orally Levodopa use for a long time has negative motor consequences.	For the desired effect, a high dose is necessary; dose failure, akinesia, and dyskinesia.
Selegiline[45]	Prevents the in vivo metabolism of dopamine Adjunctive therapy with levodopa	Therapeutic effect is mild when used alone
Amantadine[46]	Effective in reducing dyskinesia	Restlessness, depression, confusion, and hallucinations are all possible side effects.
Trihexyphenidyl Benztropine[47]	Tremors are particularly well-affected by this supplement.	Confusion, sleepiness, agitation, and delusion are all possible symptoms. Acute parkinsonian symptoms are triggered by drug withdrawal.
Ergot derivatives[48]	May be used alone to delay the need for levodopa	Causes psychiatric disorders as well as cardiovascular issues that might lead to myocardial infractions. Orthostatic hypotension, constipation, dyskinesia, disorientation, and sleeplessness are common side effects.
COMT inhibitors[49] Tolcapone[50]	It's generally used in conjunction with levodopa.	Orthostatic hypotension, dyskinesia, disorientation, and insomnia are all symptoms of sleep disturbance. Hepatotoxicity

Newly researched neuroprotective agents:

The following table summarizes the newer molecules with clinically significant neuro-protective roles in PD.

Table 3: Tabulation of newer molecules beneficial in parkinsonism therapy

Molecule	Chemistry	Role in PD
Nicotine[51]	Tobacco's alkaloid	• Nigrostriatal neurons are protected against degeneration by dopamine released from the striatum. • As free radical scavenger
Aspirin[52] Meloxicam[53]	Non-selective COX-inhibitors, COX-2 inhibitors	• Salicylic acid acts as free radical scavengers • Neuroprotective ability is independent of prostaglandin mediation
Melatonin[54]	Serotonin derivative	• Reduced generation of dopaminergic neurodegenerative hydroxyl free radicals in a dose-dependent manner
Rasagiline[55] Ladostigil[56]	MAO-B inhibitor	• Regulating and processing amyloid precursor protein
Selenium[57]	Trace metal	• Its role in the antioxidant enzyme glutathione peroxidase's action
Vitamins A, C and E[58,59]		• Lower 4-hydroxy-2,3-noneal (HNE) values • Lower 8-hydroxyguanosine levels

There is a continuous and fruitful research yield in identifying the newer therapeutic targets for the PD. The following are the list of such new targets –

☞ Potassium channels

Potassium channels – especially Kv, K_{ATP}, Kir, SK, and K2P are found in basal ganglia. These play an important role in PD pathophysiology. Drugs targeting these channels can potentially alter the behaviour and functions of the basal ganglia neurons.[60]

☞ Experimental compound, EBIO infusion into the SNc of normal mice increases the number of Tyrosine hydroxylase (marker of dopaminergic neurons) positive cells[61]

☞ In experimental models, the bee venom acupuncture prevented the loss of the dopaminergic neurons after MPTP injection.[62]

- Apamin, the main component of bee venom influences the dopaminergic pathways by persistent calcium mediated signalling. This is presumed to prevent the apoptosis of dopaminergic neurons.[63]
- Similarly, Apamin reverses the haloperidol-induced catalepsy.[62]
- Apamin treated mice in MPTP model spent less time on the rotarod test.[64]

☞ Human dopaminergic cells exposed to rotenone, when treated with experimental drug – NS309, prevented rotenone induced cell death.[65]

☞ α-synuclein

- α-synuclein accumulation is found in both alzheimer's disease and Lewy body PD.
- Understanding the mechanisms of α-synuclein mediated neuro-degeneration shall provide newer targets for the disease-modifying therapy in PD.[66]

- Inhibition of α-synuclein aggregation with small-molecules and peptide-based inhibitors is an attractive target for the drug development.[67]
- According to reports, dopamine forms a covalent bonds with synuclein and slows the conversion of protofibrils to fibrils. This suggests that the dopamine in dopaminergic neurons promotes accumulation of synuclein protofibrils, explaining the vulnerability of these neurons for degeneration.[68]
- In addition, synuclein also influences the astrocytes and oligodendroglial cells potentially playing a critical part in the aetiology of PD. This can be utilized for the discovery of novel therapeutic targets.

☞ Deep brain stimulation (DBS)

- Chronic high frequency (130 Hz) stimulation of corpus luysi (subthalamic nucleus) has shown persistent improvement in PD ranging from tremor to akinesia and rigidity. This has lead to more than 30% decrease in the drug dosage. Additionally, deep brain stimulation avoided dystonias, freezings and falls during the off period.[69]
- DBS of the subthalamic nucleus and the globus pallidus internus has been shown to be useful in the PD treatment.[70]
- Pediculopontine nucleus stimulation has shown encouraging results in management of postural instability and gait impairment.[70]
- In tremor-dominant type PD-caudal zona incerta stimulation has promising outcomes.[70]
- It is suggested that, the alternative DBS with closed loop stimulation increases the overall benefits.

☞ Striatal nitric oxide (NO)

- Synthesis of NO is by activation of NMDA and dopamine D1 receptors.
- NO diffuses into the spiny neurons of the regions and execute its actions by soluble guanylyl cyclases (sGC) receptors.
- Abnormal striatal NO-sGC pathway becomes manifested with depletion of DA. This is hypothesised to play a role in the pathogenesis of PD neuronal loss.[71]
- Overall, the net effect is loss of D2 receptor mediated inhibition of striatopallidal neurons results in exaggerated spread of cortical impulses. This uncontrolled spread of impulses may mediate some of the motor symptoms of PD.[72]

☞ Nuclear factor erythroid-2-related factor 2 (Nrf2)

- Nrf2 induces the expression of a group of cytoprotective and antioxidant enzymes.
- Nrf2 induces heme oxygenase-1, NADH oxidoreductase and enzymes of glutathione (GSH) metabolism.
- Nrf2 transcriptional activity has shown to be influenced by Catechol-derived quinones inhibit Nrf2 repressor Kelch-like associated protein to increase of Nrf2 protein levels
- Glycogen synthase kinase 3β inhibitors increase the activity Nrf2 in the nucleus.[73]
- Mixed lineage kinase (MLK)-c-jun N-terminal kinase (JNK) signaling cascade. This pathway mediates naturally occurring neuronal cell death.
- In various cell culture and animal models of neuronal death, CEP-1347, a small molecule inhibitor of the MLK pathway, has been reported to inhibit cell death.[74]

- These MLK inhibitors are potential newer molecules that can be used not only for checking the disease progression but also for reversing the neuronal cell death and preserving surviving neurons. [74]

☞ Insulin signalling

- PD and Diabetes type II share many features in disease causation and progression.
- Insulin signalling pathways are said to modulate neuronal disruption of PD.
- In the brain, neurons are proposed to undergo a process similar to the peripheral insulin resistance.
- Therapies aimed at restoring insulin signalling pathways are the novel strategy for the management of PD.[75]
- ☞ Exenatide is a GLP-1 (glucanon-like peptide-1) agonist. In a study, exenatide has shown a good results for the off-medication motor scores in PD. This class of drugs represents a new avenue in PD therapy.[76]

☞ ProNGF-p75NTR-Sortilin Signalling Complex

- The p75 neurotrophin receptor (p75NTR) is involved in neuronal survival and cell death.
- pro-nerve growth factor (proNGF) binds to p75NTR and triggers cell apoptosis. [77]
- p75NTR mediates cell death along with co-receptor ligand sortilin.[77]
- This signalling pathway is involved in substantia nigra selective neuronal loss in PD and during the disease progression. [77]

☞ Glycogen synthase kinase-3 (GSK-3)

- Regulates cell proliferation, apoptosis and development.
- GSK-3 along with insulin signalling, Wnt/β-catenin and hedgehog signalling is found to be involved in many neurodegenerative diseases including PD.[78]
- Many kinases are known to phosphorylate α-Syn and Tau proteins. Particular interest has been generated in GSK-3 as the phosphorylation of Tau and α-Syn are related to pathophysiology of PD.[79]
- GSK-3β dysregulation contributes to the parkinson's-like pathophysiology.[79]

☞Adenosine A2A receptor blockade

- These receptors are abundant in putamen and caudate.
- In animal model, A2A receptor-blockade has shown to improve many non-motor symptoms of PD. [80]
- In 6-hydroxydopamine in rat model, Anti-A2A receptor antagonists protected against dopaminergic neuronal cell death in the nigral dopaminergic neurons.[80]
- Tozadenant, preladenant, vipadenantand istradefylline, both are A2A receptor antagonists have shown promising results in the clinical trials.[81]

Many of these drugs are also tried as monotherapy for PD.[81]

☞ mGlu receptors

- Glutamate found to be increased in PD
- mGlu receptors mediate the actions of glutamate and GABA in basal ganglia.[82]
- mGlu receptor antagonists are found to be beneficial in DOPA induced dyskinesia.[82]

- This has led to the evaluation of mGlu receptors modulation for non-motor symptoms of PD.

☞ Nicotinic acetylcholine receptor (nAChR)

- α6*1 nAChRs are specific subtypes located in substantia nigra. It has limited distribution in other parts of the brain
- These receptors are located presynaptically and mediate dopamine release. [83] Its participation in a variety of motor symptoms associated with PD is being examined systematically. [83]
- As nicotine specifically protects the nigrostriatal neuronal loss, the involvement of α6* nAChRs may represent unique targets for the therapeutic management of PD.[81]

☞ Protein kinases CK1 and CK2

- Second most important group of drug targets
- Ser129 of human α-synuclein is phosphorylated by casein kinase 2, and is the major alteration before the accumulation of these changed proteins in α-synucleinopathies. [84]
- A novel inhibitor of CK2 is 1-(Benzo[d]thiazol-2-yl)-3-phenylureas has been tried for the neurodegenerative disorders including PD.[85]

Drugs discontinued from research in parkinsonism therapy:

☞ Merck discontinued its clinical trial using preladenant.

- Preladenant is an adenosine A2A receptor antagonists.
- Preladenant was shown to be unsuccessful in a phase III trial, and the drug was withdrawn from the market.[86]

☞ Contrary, istradefylline and tozadenant are showing some promising results and the phase III trials are on.

Table 4: Newly approved drugs for parkinsonism

Drug	Mechanism of action	Comments
Safinamide[87]	Selective monoamine oxidase B inhibitor. Also inhibits glutamate release and dopamine, and serotonin reuptake	Adjunct therapy with levodopa/carbidopa
Istradefylline[88]	Selective adenosine A2A receptor inhibitor	Adjunct therapy with levodopa/carbidopa Treatment improves "off" time when used as add-on

Non-pharmacological treatment

Table 5: Tabulation of non-pharmacological therapy beneficial in parkinsonism

Novel therapy	Currently available	Future therapy and comments
Gene therapy[89,90]	Glial cell line-derived neurotrophic factors (GDNF)	Adenoviral and lentiviral nigrostriatal implants to liposomes
Surgical methods		
Pallidotomy[91]	Destruction of pallidum by electronic probe	Surgery may be considered for patients who are suffering from unacceptable side effects.
Thalamotomy[92]	The thalamus is a part of the brain that is removed.	Rarely performed
Thalamic stimulation[93]	An electrode wire is inserted into the thalamus.	Effective in the management of tremor
Deep brain stimulation [DBS][94]	Implanted electrode in the brain	Prevents impulse transmission

Herbal medicines effective in Parkinsonism therapy

Many herbal medicines have shown to be beneficial in the management of PD with improvement in symptoms of both motor and non-motor. However, their efficacy as monotherapy in PD treatment remains a question for debate.[95,96]

There is renewed interest in testing plant extracts for the PD treatment. The following plant extracts in various setting were found beneficial in management of PD.[97]

- Tinospora cordifolia
- Sesame seed oil
- Carthamus tinctorius
- Chaenomeles speciosa
- Portulaca oleracea
- Paeonia suffruticosa
- Mucuna pruriens
- Hyoscyamus niger seeds
- Hibiscus asper leaves
- Gynostemma pentaphyllum
- Ginkgo biloba
- Fructus Alpiniaoxyphylla
- Delphinium denudatum
- Bacopa monniera Linn
- Althaea officinalis
- Albizia adianthifolia
- Valeriana officinalis
- Black tea
- Panax ginseng
- Safflower

Background of the study in detail

The documented neuronal change in parkinsonism is the gradual degradation of dopaminergic neurons in the substantia nigra-pars compacta. Neuroinflammatory processes accelerate the loss in the neurons and the oxidative stress injury, which leads to changes in the mitochondrial membrane permeability, enzyme metabolism, and mitochondrial genome modifications.[5] In addition to these methods, the brain renin-angiotensin system (RAS) has been shown to influence the learning and memory functions of the brain, maintaining body water balance, blood pressure, sexual behaviour, and pituitary glandular secretions. [98] This RAS system in the brain is implicated in the pathogenesis of neurological diseases such as alzheimer's disease[99] and PD.[35,100]

Angiotensin II acts on the certain areas of the brain influencing the drinking behaviour and natriuresis.[101] It stimulates the vasopressin release, modulates the sympathetic outflow and decreases the baroreceptor reflex.[102] It is postulated that the most of these effects are through AT1 receptors. Animal studies have added the necessary evidence to the notion that AT1 receptor influences the cell proliferation, water intake and blood pressure.[102,103] Angiotensin II can stimulate the catecholamine release through AT1 receptor stimulation.[104] Rodent studies have also shown that the angiotensin receptor binding in substantia nigra-pars compacta, and bring about presynaptic effects in the dopaminergic neurons in the region.[35,105, 106]

Studies have shown that losartan, an AT1 receptor antagonist, protects dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity in primary ventral mesencephalic cultures and the substantia nigra-pars compacta of mice.[107] According to a few interventional trials, losartan has shown a promising role in neuroprotection[108] in atypical parkinsonism. Losartan has been

found to protect dopaminergic neurons in the midbrain from the death caused by rotenone.[109] Candesartan, another AT1 receptor blocker, has shown a promising role in a rotenone rat model of PD.[110] The neuroprotective role of losartan in the rotenone rat model, however, has yet to be established.

Even though there are few reports of captopril inducing parkinsonism,[111,112] there are many series of studies proving protective role of captopril in parkinsonism at least in the animal models.[22] When used to treat arterial hypertension in parkinsonism, latest studies have shown that the captopril causes insignificant drug interactions with bromocriptine and cabergoline.[113] This prompted us to earnestly evaluate the neurobehavioral effects and neuroprotective characteristics of these widely used antihypertensive drugs, captopril, perindopril and losartan.

There is renewed interest in evaluating the positive benefits of angiotensin converting enzyme (ACE) inhibitors such as perindopril as curiosity and understanding of the brain renin-angiotensin system increase. Much research had documented its neurobehavioral benefits and neuroprotective properties. Its use in the management of motor fluctuations and dyskinesia associated with PD had been partially validated clinically.[114]

Therefore, with these three drugs acting on the RAS, we intended to evaluate the beneficial role in parkinsonism. During the study, an attempt had also been made to evaluate the neuroprotective roles of these drugs with respect to oxidative stress induced neuronal loss.

Need of the study

Drugs used in the parkinsonism therapy centralises on the motor symptoms. Current therapies do not address the disease causation and progression. Issues of neuronal protection and the endurance of dopaminergic neurons are seldom addressed with the current therapy. The principal drug, L-dopa has highly limited potential of addressing non-motor component of PD.

Newer targets for PD treatments have emerged as a result of better knowledge of the brain renin-angiotensin system and its connection with the hepatocyte growth factor (HGF) and c-Met receptor systems. Vasoconstriction, neuroinflammation, oxidative stress, and apoptosis are all influenced by angiotensin I, II, III, and IV. They do so by engaging AT1 receptors via angiotensin 1-7 and angiotensin 3-7 subsidiaries. On the other hand, angiotensin derivatives that operate on AT2 and AT4 receptors have been found to cause angiogenesis as well as anti-inflammatory, anti-oxidative, and anti-apoptotic effects.[115 - 118]

These varied spectrums of changes, the drugs acting on the brain angiotensin system can influence the very pathogenesis and progression of the PD. Worldwide, researchers are making sustained and major efforts to determine the positive impact of angiotensin receptor blockers and angiotensin converting enzyme inhibitors in the treatments of PD.

With the growing evidence of neuro-inflammation as one of the important component of neuronal loss, it is a pre-requisite for any animal model to evaluate the inflammatory process in order to establish any molecule or to extract with anti-parkinsonism effects.

Parkinsonism treatment and management: problem statement

Overall, more than 6.2 million people are living with parkinsonism in the world (global burden of disease 2015, neurological disorder collaborator group).[119] More than 117,400 death are accounted by parkinsonism globally. PD is the disease of elderly. Approximately 1% of the all people aged more than 60 years have PD. It has male predominance. PD in a people less than 50 years is called young-onset PD.[120]

According to the World Health Organization, the "estimated crude prevalence" (the total number of old and new cases per year) is 160 per 100,000, and the "estimated incidence" (the number of new cases each year) is 1619 per 100,000.[121] The prevalence of PD varies around the world. North America and Europe are thought to have higher rates of PD than Asia and Africa.[122] However, studies have been conducted to determine the causes of PD and the use of medicinal plants in the treatment, prevention, and cure of the disease.

Despite living in a country, the Parsi community in Mumbai has the highest prevalence of PD in the world, with roughly 328 out of every 100,000 people affected.[121, 123] In comparison to many other countries, India has a lower prevalence of PD (70 cases per 100,000). Albania has the highest frequency of PD (800 per 100,000).[124] With a prevalence of only 7 per 100,000, Ethiopia has the lowest recorded prevalence of PD in the world.[125] PD affects almost 1.7 million people in China alone.[126]

Men suffer from PD in greater numbers than women. The ratio of males to females with PD, on the other hand, varies a lot according to the country. In Nigeria, men have PD[17] in considerably greater numbers than women. In Japan, PD affects more women than men.[127]

With such a high prevalence numbers, the research interest in aetiology, pathogenesis, progression of the disease, treatment and management of associated conditions are preeminent. Research interest in India regarding PD matches with any other developed country with more than 452 published articles in PubMed till 2014.[18] This meta-analysis excludes all the animal studies and mainly clinical trials included. Out of this, most research papers address the clinical manifestations and genetic concerns of PD. Among all the research institutions, only three centres monopolize the PD research, namely, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, All India Institute of Medical Sciences (AIIMS), New Delhi and Bangur Institute of Neurosciences/ Anthropology Society of India, Kolkota.[18]

Current hypothesis of parkinson's disease

Parkinson's disease[PD] is caused by dopaminergic neuron degeneration in the substantia nigra-pars compacta.[128] Insufficient activation of striatal dopaminergic D1 and D2 receptors results from the loss of dopaminergic neurons.[129] Bradykinesia, resting tremors, and rigidity are the symptoms of low dopamine levels. These three signs and symptoms are the classic motor manifestations of PD.

Evidence is building up to suggest that PD result from reactive oxygen species mediated neuronal loss from a neuro-inflammatory process.

L-dopa is effective for the control of motor symptoms, but do not completely address the non-motor problems.

Present day therapy of PD include - relieving symptoms of PD with dopamine (DA) replacement. To protect the dopamine produced, L-dopa, DA receptor agonists, monoamine oxidase B inhibitors, and catechol-O-methyltransferase inhibitors are being used.[21, 130]

Reduced mobility, dyskinesia and spontaneous involuntary movements complicate PD care as the disease advances. The progression of symptoms is attributed to the gradual neuronal loss, which includes noradrenergic, cholinergic and serotonergic neurons in addition to the dopaminergic neurons. In the later stages of the disease, non-motor symptoms such as depression, dementia, and autonomic nervous system disorders may become more apparent.[19, 27, 28]

Additional to L-dopa, apomorphine,[131] surgical interventions like deep brain stimulation[132] and pallidotomy[133] are advocated for the patients who continue to have severe motor symptoms with the disease progression.

Currently, researchers are concentrating on the development of newer techniques for stopping the neuronal loss, neuroprotection and evaluating the brain renin angiotensin system for the control of motor and non-motor symptoms of PD. Overall, slowing or reversal of dopaminergic neuronal loss resulting in the betterment of motor and non-motor function is the essence of current research.[26]

Renin-angiotensin system – newer target for Parkinsonism therapy

The majority of drugs used to treat PD focus primarily on the symptoms. Current treatment methods rarely address neuroprotection and preventing dopaminergic neuron degeneration. L-Dopa is the most effective therapy modality for PD when it comes to regulating motor symptoms. Non-motor symptoms, on the other hand, are largely unaffected by this drug.[27] At the same time, L-Dopa and its metabolites, dopamine, are toxic to the remaining dopaminergic neurons in the substantia nigra. Despite the fact that there are significant methodological discrepancies in the research papers attributing the detrimental end outcomes to L-Dopa, it is still the most commonly prescribed drug for reducing the motor symptoms.[21] Alternative therapeutic techniques are being sought, notably for those

involving dopaminergic neurons in the substantia nigra. There are three main alternative therapy techniques that are now popular.

1. The renin-angiotensin system in the brain
2. Interactions between neurotransmitter systems and the hepatocyte growth factor (HGF)/c-Met receptor system
3. Angiotensin IV and the HGF/c-Met System Interaction

The brain renin-angiotensin system is detailed in this study as a result of these differing perspectives. HGF, commonly known as "scatter factor," was discovered to enhance liver regeneration after being extracted from the liver.[115] As per the studies, HGF levels in the cerebrospinal fluid have been found to be increased in people with PD. Based on these findings, it is suggested that HGF-targeted compounds to be used to treat neuroimmune disorders[116, 117] and neurodegenerative diseases,[134] such as parkinsonism. The study also revealed that the HGF/c-Met system functions coincide with those of angiotensin IV. Memory consolidation, neuronal development, calcium signalling, dendritic arborisation, and cerebrospinal fluid physiology are mediated by them.[135] Aside from their usual roles, they've been linked to neuroprotection, seizure control, and wound healing.[136] As a result, it's thought that angiotensin IV analogues work by activating the HGF/c-Met pathway. As a result, Norleual-AngIV, an angiotensin IV receptor antagonist, suppresses HGF binding to c-MET, as well as HFG-dependent signalling, proliferation, invasion, and scattering.[118]

Link between Parkinsonism and the brain's angiotensin system

Allen and colleagues discovered angiotensin receptor binding sites in the corpus striatum and the substantia nigra pars compacta of the midbrain, both of which contain dopamine-containing cell bodies.[137] They discovered that ACE is abundant in striosomes in striata and is found in the substantia nigra-pars reticulata. They discovered a reduction in the angiotensin receptor binding in the substantia nigra and the corpus striatum of post-mortem brains from PD patients using autoradiography methods.[137] Based on the findings, they hypothesised that the drugs that interact with the angiotensin system, especially angiotensin converting enzyme inhibitors and angiotensin receptor blockers, would regulate the dopamine system in the brain. Numerous subsequent studies.[138-140] had shown the presence of ACE in the nigra-striatal pathway and basal ganglia tissues. Furthermore, ACE's role in the metabolism of bradykinin has been well-established for nearly three decades.[141] Bradykinin is a factor in PD.[26] Furthermore, ACE has been demonstrated to metabolise bradykinin and thus, the inflammation.

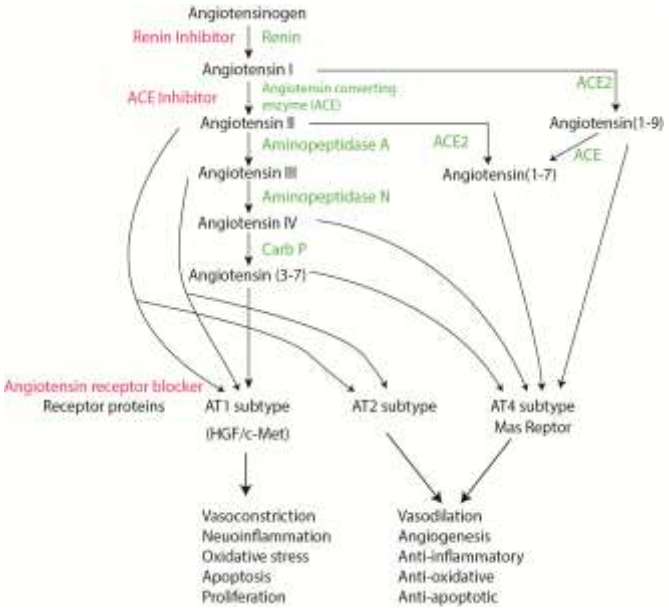

Figure 4: Schematic representation of renin-angiotensin pathway with active ligand, enzymes (green), receptors and drugs influencing (red)

Moreover, AT1 receptor subtype activation by angiotensin II lead to NADPH (nicotinamide adenine dinucleotide phosphate) – dependent oxidases. This lead to the significant oxidative stress related neuronal injury.[141-143]

None the less, brain RAS has no straight forward effects on the neurobehavioral effects. Angiotensin II, an octapeptide has the disruptive effects on the learning and memory, whereas angiotensin IV, a hexapeptide facilitates the memory acquisition and consolidation. Better knowledge of local (brain) RAS system and its components lead to a specific targeting of the receptors with more specific clinically beneficial effects. In addition to all this, recently specific renin receptor and its precursor prorenin were also reported.[144] Researchers had hypothesized that the brain angiotensin II levels are higher than the circulating levels. All the components of RAS that were reported in the periphery are accounted in the brain including ACE, angiotensin II, III and IV receptors present in the brain.

Evidence of brain angiotensin system involvement in Parkinsonism

A number of studies had found evidence of a relationship between RAS in the brain and PD. The use of ACEIs and ARBs is related with a lower incidence of PD, according to a nationwide cohort study evaluating the use of anti-hypertensives in more than 65 thousand patients with PD in Taiwan.[145] Other anti-hypertensive drugs, on the other hand, may not always result in a reduction in the incidence of PD. Calcium channel blockers might not be effective in preventing PD.[146] There is little research to back up the claim that calcium channel blockers could reduce the risk of PD in hypertensive patients.[147- 148]

Patients with PD who were given an ACE inhibitor, perindopril, at the same time saw an improvement in their motor symptoms.[114] After analysing 60 PD patients who were taking an ACEI for hypertension, Loudisio et al. concluded that an ACEI

may be independently associated with a lower risk of falling and a lower number of falls in individuals with PD.

Numerous researches had proven perindopril's therapeutic effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of PD. According to Sonsalla et al.,[22] captopril protects the striatum from MPTP neuronal damage and continuous administration of captopril protects dopaminergic neurons from degeneration in rats. According to Muoz et al., captopril, in addition to influencing the motor coordination of MPTP-induced parkinsonism, also reduces oxidative stress. They believe that inhibition of angiotensin-activated NADPH-dependent oxidases has this effect on oxidative stress.[149] Katrina et al. found that a four-week course of perindopril improves the clinical characteristics of PD by reducing "on-phase" dyskinesia.[114] Perindopril's neuroprotective benefits have also been demonstrated in the rat MPTP model.[150] According to one study, the combination of aspirin and nimodipine improved the neuroprotection and the motor symptoms in rats using the MPTP model.[52] Recent research has linked brain RAS to other neuronal diseases, including stress and anxiety,[151] depression, cognitive dysfunction,[152] and alcohol consumption.[153] AT1 receptor inhibition has been linked to improved learning, spatial memory, and motor coordination.[154, 155] Overall, a Cochrane review article concluded that there is an insufficient evidence to recommend the use of antihypertensive drugs for either primary or secondary prevention of PD.[156] The authors of this review had called for the greater research to determine the role of ACEI and ARBs in the treatment and prevention of PD. With the angiotensin system being implicated in the progression and clinical manifestations of PD, it was chosen to evaluate the positive effects of two ACEIs, captopril and perindopril, and one ARB, losartan, in this study. Efforts had been made to see how these three drugs affect neuro-behavioral impacts,

oxidative stress, and neurotransmitters & inflammatory marker in the brain after they were administered. We primarily wanted to notice the impact of these drugs on the brain in terms of histo-architectural changes, and effects as demonstrated by immunohistochemistry for apoptic cell death during this process.

Rationale behind selection of Parkinsonism models

Rotenone model

More precise reproduction of human PD is possible with the systemic rotenone administration in the rats.[157] The rats with rotenone induced parkinsonism have bradykinesia, rigidity and postural instability. These manifestations can be reversed by the administration of apomorphine. These laboratory manifestations are more consistent with the nigrostriatal dopamine system. Greenamyre and Cannon group had popularized this method of parkinsonism worldwide.[157] Johnson et al had shown for the first time that the rotenone model could reproduce the two important hallmark effects of PD, namely motor deficits and extranigral effects,[158] and interestingly the accumulation of endogenous alpha-synuclein. Rotenone could cause ATP depletion, oxidative damage, and death of dopaminergic neurons in the substantia nigra-pars compacta in a dose-dependent manner.[159]

Rotenone enters the cell by diffusion. Mitochondrial transmembrane potential is altered after its entry into the mitochondria. Rotenone, then inhibits the mitochondrial complex I and microtubule formation.[159] This leads to the neuronal damage.

Many pesticides contain rotenone as a component. As a result, interest in rotenone as a risk factor for the development of human PD is growing.[160] Two studies that had shown that the rotenone has a substantial causal relationship with PD.[29, 30] The main limitations of this mitochondrial poison, rotenone model is the dose –

dependent toxicity leading to a variable induction of symptoms and mortality.[31] There are also reports of severe gastrointestinal problems and other milder systemic toxicity in the animals.[161]

In spite of these limitations of rotenone model, we had adopted this model because this model would surely lead to a precise parkinsonism induction with motor, non-motor and extranigral manifestations.

MPTP model

From days of accidental discovery of MPTP among "synthetic heroin" users in multiple northern Californian towns, this compound has been established to selectively destroys the dopaminergic neurons in the substantia nigra-pars compacta.[162] The effects were unambiguously proven among the non-human primates.[38,163,164] Monkeys have almost similar motor symptoms as that of humans.[165,166] MPTP also shown to be experimentally effective in inducing selective degeneration of dopaminergic neurons in other animals like salamanders,[167] zebra fish[168] and C. Elegans.[169]

There are more than seven thousand studies in the last two decades evaluating the effects of MPTP in rodents.[162] Both mice and rat are equally susceptible to MPTP. Of late "model fusion" approach also been tried. In the model fusion, two or more factors influencing the effects or preventing the effects of MPTP are combined.[170] The outcome measures of the fused model can include the effects of MPTP as well as the other factors simultaneously.

When MPTP is mixed with normal saline and given to the animals intraperitoneally, the central and peripheral monoamine oxidases-B convert the MPTP to MPP+ radical (MAO-B).[171] Dopamine-producing neurons of the substantia nigra-pars compacta deteriorate as a result of these radicals. As a result of the death of

dopaminergic neurons, this process eventually leads to parkinsonism in a short period of time. We chose the MPTP model for the evaluation of the drugs in this study since it is the most regularly used animal model for evaluating the effects of experimental pharmaceuticals, molecules and plant extracts.

The induction and manifestation of parkinsonism is better appreciated in the mice than the rat.[23] Therefore, mice model of MPTP was used to evaluate the beneficial effects of the drugs in the present study.

Paraquat model

One of the most extensively used herbicides is paraquat. This molecule's chemical name is N,N′-dimethyl-4,4′-bipyridinium dichloride. It has a structure that is almost identical to MPP+. Pests in soybeans, sorghum, sugar cane, cotton, corn, and apples are widely controlled using this broad-spectrum herbicide.[172] Many epidemiological studies have found that combining paraquat exposure with other factors such as rural life, farming, and well water intake increases the risk of developing PD.[20,29,32] When given intraperitoneally or orally, paraquat dramatically reduces motor activity in mice, which correlates with a decline in the dopaminergic neurons in the substantia nigra-pars compacta.[173] The production of phosphorylated-synuclein in the enteric nervous system of young mice is triggered by the intraperitoneal administration of paraquat. In mice, the induction and manifestation of parkinsonism are better understood than in rats.[23] As a result, we used a paraquat model in mouse for our research to assess the positive effects of the drugs. The Na+ dependent neutral amino acid transporter allows paraquat to pass through the blood-brain barrier.[174] Paraquat enhances NADPH reducing equivalents by hijacking the pentose phosphate pathway.

In addition, it stimulates the redox cycling.[175] This leads to the impaired recycling of glutathione and thioredoxin leading to the inhibition of intracellular antioxidant system.[173] The apoptosis end results of paraquat is attributed to the up regulation of Bcl-2 family proteins leading to cytochrome C release and the activation of caspase 3.[176] Even a single dose of administration of paraquat can result in nearly 50% loss of dopaminergic neurons in the mice. It's specificity to substantia nigra is mediated through microglia.[177]

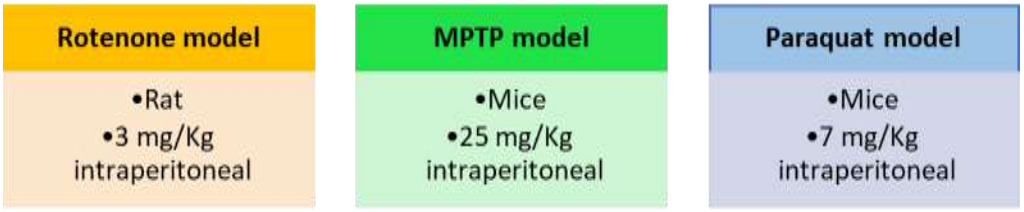

Figure 5: Summary of animal model selected for the evaluation of three drugs during the study

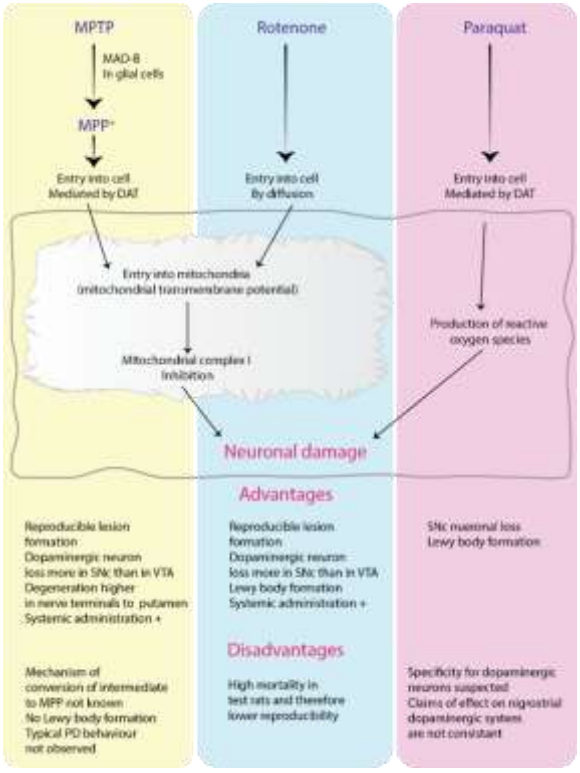

Figure 6: Schematic representation of neuronal damage in three animal models selected in the present study

Rationale for selection of outcome measures

The following outcomes were measured in this present study:

- Neuro-behavioural changes
- Oxidative stress measurement
- Changes in the neurotranmitters & inflammatory marker levels
- Histo-anatomical changes in various parts of the brain
- Immunohistochemistry

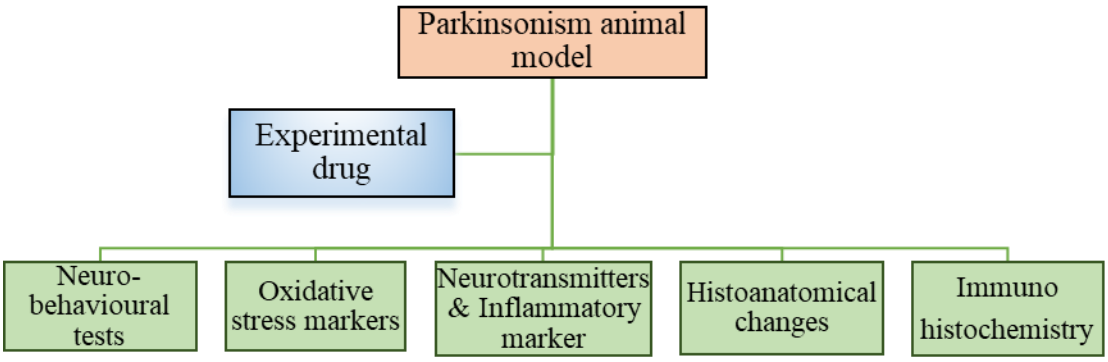

Figure 7: Schematic representation of outcome measures of the study

Selection of neuro-behavioural models

The purpose of these neuro-behavioral analyses was to concentrate on the impact of these three drugs on the angiotensin system in the brain. Therefore, all the models that quantify the motor functions, exploratory behaviour, depression and anxiety like manifestations were carefully considered, and thus, only those models that met with the objectives were short-listed.

There might be wide variations in the degree to which the PD can be induced in the rodent model with above selected models (Rotenone, MPTP and Paraquat). To add to this variability, the models quantifying the motor behaviour too have inherent variability. While selecting the behaviour tests, it may be undermined that while the patients experience wide range of symptoms ranging from akinesia, bradykinesia,

muscular rigidity, dystonia, resting tremors, gait abnormalities, postural instabilities to non-motor symptoms, rodent model falls a way short of this spectrum of clinical manifestations.[178] Although the many animal models exactly replicate the dopaminergic neuronal loss in substantia nigra, the resultant laboratory manifestations and subsequent quantifications may not have completely overlapping and reproducible outcome measures. Therefore, there are many behavioural tests advocated to quantify the effects of the neuro-toxins and intern the experimental drug/molecule in question.

To assess the learned and / or innate motor skills, rotarod test, grip test, inclined beam traversal, forelimb placing test, adjusting steps, climbing down a pole, reaction-time test, paw retraction test, staircase test, nesting behaviour and adhesive removal are advocated.[179,180] There are variable association of these tests/models with the dopaminergic neuronal loss and degree of detection of motor impairment. Among these learned behaviours, skilled forepaw actions, including placing as happen with forelimb placing test, adjusting down a pole, grip test, have clear correlation with PD.[178] Therefore, these forelimb adjustment tests are primarily advocated for the evaluation of the motor impairment following toxic loss of dopaminergic substantia nigra-pars compacta neuronal loss.[181]

Spontaneous locomotor activity, the rota rod test and the grip-strength test were used to assess motor functions. The hole board test was used to assess exploratory behaviour. The effects of depression on behaviour was investigated using a forced swim test and a tail suspension test. The elevated plus maze test was used to determine the effects of anxiety on behaviour.

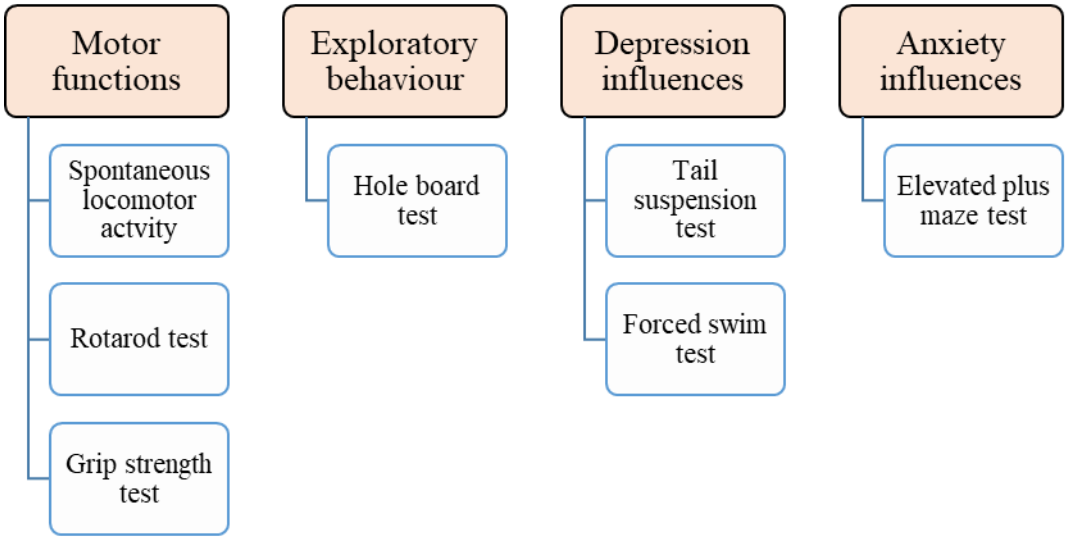

Figure 8: Summary of behavioural tests selected during the study after induction of Parkinsonism among rodents

Spontaneous locomotor activity: Actophotometer measurements of spontaneous locomotor activity provide an accurate estimate of overall motor functioning. The number of counts every ten minutes was used as a measure of locomotor activity

Rota rod test: The amount of time (duration) an animal remains upright on a rota rod without falling is a measure for their coordination, balance, physical condition and motor planning ability.

Grip strength test: Each animal was held on a thread [at a height of 50 cm] with their front paws to assess grip strength, and the time in seconds was recorded.

Hole board test: Increased exploration of the holes was indicated by a decrease in the anxiety on the hole board test.

Tail suspension test: In a tail suspension test, the rodents' tails were suspended them above the ground. With their tails dangling, the animals' movement increases, indicating less depressed behaviour.

Forced swim test: The amount of time a rat spent immobile in water was used to determine the depression-like behaviour.

Elevated plus maze: The number of entries into the open arm, the number of entries into the closed arm, and the duration spent in the open arm were used to indicate the animal's anxious behaviour in an elevated plus maze test.

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Selection of oxidative stress markers

With oxidative stress-induced dopaminergic neuronal loss emerging as a more significant etiological factor, it is critical to assess the extent of free radical-induced brain injury during toxic rodent model testing. Oxidative stress markers are molecules that undergo changes as a result of interactions with reactive oxygen species or as a result of an enhanced redox state. Reactive oxygen species have the potential to damage all of the DNA, lipids, and proteins in the brain. This can have negative consequences ranging from altered neuronal functions to cell death.

Superoxide dismutase breaks down superoxide into non-reactive oxygen species. It is present in all the cells that are exposed to oxygen. It neutralizes the toxic free radicals. Decrease in the SOD indicates the oxidative stress environment.

Proteins can be oxidatively altered in one of two ways. The nitration reaction is mediated by protein tyrosine kinase, which produces peroxynitrite (ONOO-). This forms oxo-metal complexes and nitrogen dioxide when it reacts with myeloperoxidase. Later on, it helps with the nitration reaction.[182] The creation of a disulfide bridge between cysteine and glutathione is known as S-glutathionylation. The endothelial nitric oxide synthase, ryanodine receptor and sodium potassium pumps are all affected by this oxidative alteration.[183]

Catalase is an enzyme that reduces the dangerous levels of hydrogen peroxide in the body. Hydrogen peroxide is converted to water and non-reactive oxygen species. As a result, it prevents the generation of free radicals from peroxide. Catalase levels are reduced in oxidative stress.

Lipid peroxidation plays an important role in free radical-mediated neuron damage.[184] Because of the abundance of double bonds in their structure, lipids are prone to oxidation.[185] Malondialdehyde and isoprostanes are two of the most commonly researched oxidative stress markers. Lipid hydroperoxides and oxysterols are two others.[183]

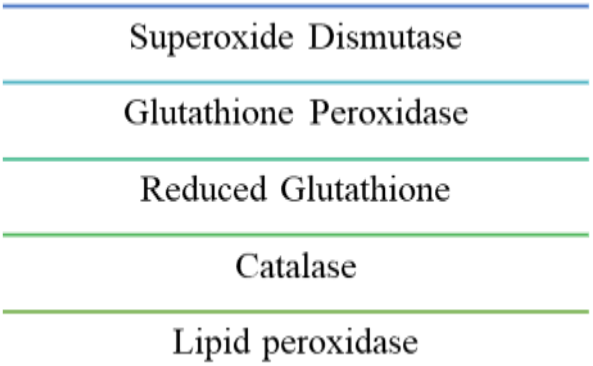


Figure 9: Markers selected in the study to estimate the oxidative stress

Selection of neurotransmitter evaluation

Serotonin

Of late, there is a notion that parkinsonism is much more than dopaminergic neuronal loss manifesting as motor symptoms alone. Brain damage in the PD starts much before the motor symptoms manifests. In a study by Morios Politis et al, through PET scans had shown that in the midbrain area has reduced serotonin transporters in patients who were carriers of PD–causing mutations.[186] Subsequently, many researchers had successfully documented the serotonergic loss concurrent to the dopaminergic neuronal loss.[187,188] This potentially challenged the dopaminergic theory behind the parkinsonism.

Moreover, serotonin is directly implicated in the mediation of dyskinesia that appear during the disease progression.[189,190] Various serotonin receptors perform neurologically diverse actions and the behavioural outcomes in the normal

individuals. The serotonin receptors in the prefrontal cortex are said to influence the cognition and the motor executive functions in the neurodegenerative disorders including alzheimer's disease and parkinsonism.

Therefore, in this study, we selected serotonin evaluation in the homogenized brain tissue. This was expected to delineate deeper details in the neurotransmitters involved in the parkinsonian rodent model and to demarcate the possible underlying protective effects of the angiotensin system affecting drugs studied.

Dopamine

With the loss of dopaminergic neurons of substantia nigra-pars compacta, many human experiments backed by thousands of animal experiments agree that there is a decrease in the overall dopamine levels in the region. This is true irrespective a small area of midbrain ventral to tectum[191-193] is evaluated or few sections of midbrains[159,191] were considered or entire brain tissue is homogenized and overall dopamine levels[194] were evaluated. This was also equivocally proven in the unilateral localized toxin induced parkinson's rat model by many studies.[195] Such hemi – parkinsonism models are more objective and authentic.[196]

GABA

GABA (Gamma-aminobutyric acid) is the chief inhibitory neurotransmitter in the brain. Similar inhibitory actions are also seen in the peripheral nervous system and the enteric nervous system. In the pathogenesis of PD, the following symptoms are proven to have direct or indirect relation to GABA.[1897]

- Dysautonomia, gastrointestinal disturbances, constipation – GABA mediated inhibition of dorsal nucleus of vagus

- Glossopharyngeal control deficits – GABA mediated influences on the nucleus ambiguous.
- Anxiety and sleep disturbances - lack of GABA in the locus ceruleus and hypothalamus leads to a progressive deficiency of the noradrenergic, serotonergic and dopaminergic systems.
- Bradykinesia and akinesia are associated with an increase in the threshold for GABA spiny neurons in the striato-pallidal complex.
- Hypomania - GABA deficit and dopaminergic neurodegeneration, as well as glial-based synaptic dysfunction in the mesolimbic and nigrostriatal dopaminergic systems.
- Stiffness, tremors, bradykinesia and postural instability are caused by decreased spontaneous GABA activity in the striato-pallidal complex.

When GABA receptors are activated, neurons become hyperpolarized, inhibiting synaptic transmission for a long time. At the molecular level, the calcium/GABA pathway usually stabilises neuronal activity. The breakdown of this inhibitory system causes irreversible brain ageing and neurodegeneration. In addition, GABA inhibition withdrawal leads to the vasodilation. This leads to an increased permeability and changes in the blood brain barrier, more inflammation and intensified neuronal damage.[198]

Therefore, it was prudent to evaluate the GABA levels in the brain homogenates after induction of PD in the rodent model and during neuro-protective evaluation of the three drugs acting on the brain angiotensin system (namely captopril, perindopril and losartan).

Glutamate

Dopaminergic neurons that have been damaged are sensitive to glutamate's actions. When cellular energy metabolism is disrupted, glutamate can become neurotoxic. As a result, glutamate plays a role in the development of PD. When dopaminergic neuronal denervation occurs, the activity of basal ganglia nuclei undergoes a series of functional changes.[199] Dopaminergic neurons become sensitive to oxidative stress in two key situations.[200,201]

- Mitochondrial dysfunction caused by a deficiency in complex-I
- Dopamine oxidation and the resulting oxidative stress in neurons

A balance between excitation and inhibitory activities is also maintained by neuronal and astrocyte networks. In a calcium-dependent manner, astrocytes absorb glutamate and produce GABA. GABA is recycled back to glutamate via the tricarboxylic acid cycle. This glutamate-GABA-glutamate recycling mechanism maintains a healthy balance of excitement and inhibition.[197] Because there is a direct and indirect evidence linking glutamate to the pathogenesis of PD in humans and the induction of PD in toxin animal models, measuring glutamate in homogenised brain tissue was crucial in assessing the neuro-protective effects of the drugs that were studied (captopril, perindopril, and losartan).

Acetylcholine

Acetylcholine is a ubiquitous molecule, having pivotal role in many parts of the CNS and PNS. PD association with acetylcholine is not recent. There are many symptoms of PD resulting due to the involvement of nicotinic and muscarinic cholinergic receptors.[202]

- Many of the motor symptoms are accounted due to the altered cholinergic striatal tone
- Gait impairment and falls are partially due to the degeneration of nucleus basalis magnocellularis (Meynert's nucleus) and pedunculopontine nucleus
- Cognitive impairment is also attributed to the degeneration of nucleus basalis magnocellularis
- Sleep behavioural changes in PD are possibly due to the degeneration of pedunculopontine nucleus
- Psychosis in PD is attributed to the reduced cholinergic tone

Neuroprotection can be achieved in PD by using drugs acting on the nicotinic receptors. anticholinergics were the first groups of drugs used in the therapy of PD and they are still finding the place in both as monotherapy and in combination with other drugs. Many clinical trials have established the useful effects of benzhexol, orphenadrine, benztropine, bornaprine, benapryzine and methixine.[203] Donepezil, galantamine, rivastigmine, tacrine and trichlorfon are found clinically effective in the symptomatic improvement of PD.[204, 205]

With this proven background of association of acetylcholine in both the pathogenesis and therapy, it was prudent to analyse its levels while evaluating the effects of drugs (captopril, perindopril and losartan) acting on the brain angiotensin system for potential therapy for PD in the animal models.

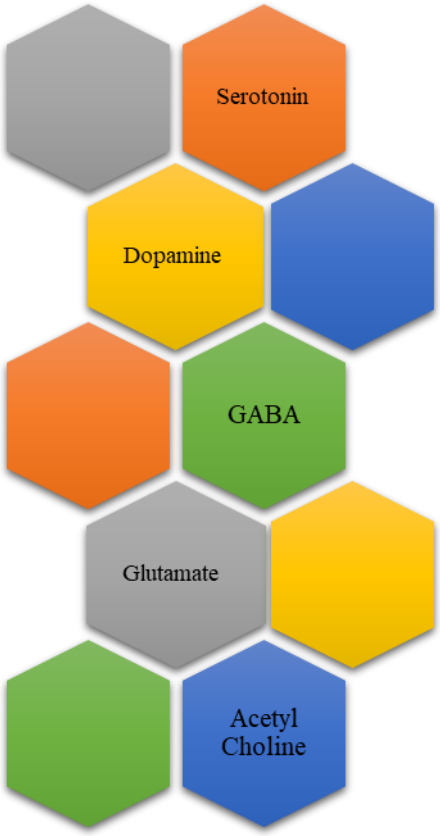


Figure 10: Schematic representation of neurotransmitters assayed during the study

Selection of inflammatory marker

Myeloperoxidase [MPO]

Granules containing myeloperoxidase are abundant in active neutrophils, macrophages and monocytes (MPO). It is feasible to generate reactive oxygen species rather quickly by catalysing the conversion of hydrogen peroxide to OH, ONOO, hypochlorous acid (HOCl) and NO_2. Lipids, lipoproteins and proteins can all be altered by these reactive species. The peroxidase activity of MPO was also evaluated using a UV spectrophotometer.

Rationale for the histo-anatomical changes in PD

As is widely known, PD is caused by the death of dopaminergic neurons in the substantia nigra pars compacta of the midbrain. The accumulation of presynaptic

protein-synuclein or the microtubule binding protein tau are the most common pathologic abnormalities in PD. The accumulation of α-synuclein in the neuronal perikarya causes formation of Lewy bodies, and the accumulation of α-synuclein in the neuronal processes causes Lewy neurites.[206,207] The majority of motor symptoms are linked to these pathological alterations. Tau proteins are found not just in neurons but also inside glial cells in progressive supranuclear palsy. This impairment, often known as 'Parkinson plus' disorder is linked to postural instability (which can lead to an early fall), dementia and eye movement dysfunction.[208] Multiple system atrophy is a pathological disorder that affects not only nigrostriatal dopaminergic pathways but also pontocerebellar and olivocerebellar fibres. Multiple system atrophy is characterised by the presence of α-synuclein in the cytoplasm of oligodendrocytes. Glial cytoplasmic inclusions are a specific type of glial cytoplasmic inclusion.[209] The loss of dopaminergic neuronal loss in the substantia nigra-pars compacta coincides with the classic triad of bradykinesia, tremors and rigidity. The cerebral cortex, locus ceruleus, vagal nucleus, Meynert's nucleus, olfactory nerves, sympathetic ganglia and myenteric plexus have all been found to contain α-synuclein. Not only dopaminergic pathways are affected, but also serotonergic, norepinephrine and cholinergic pathways.[187,189] With a rising interest in the study of the gut-brain axis, there is mounting evidence that α-synuclein aggregation begins in the enteric nervous system, and then travels to the brain via the olfactory tract and vagus nerve. This is regarded as "Braak hypothesis."[210]

The pathophysiology and spread of Lewy bodies

The appearance of Lewy bodies is hyaline or shiny. The cytoplasmic inclusion of α-synuclein is pale and poorly defined. [206] Pale bodies are cytoplasmic inclusions that are pale in colour and found in the substantia nigra and locus ceruleus. The

appearance of Lewy bodies precedes the production of pale bodies, called “pre-Lewy bodies”.[211] Neuronal loss, extraneuronal neuromelanin pigment, and gliosis in the substantia nigra are all symptoms of PD. In postmortem investigations of parkinson's brains, consistent pathological alterations have been found in the hippocampus, amygdala, basal nucleus of Meynert, corpus striatum, hypothalamus, substantia nigra and medullary tegmentum (containing the dorsal motor nucleus of vagus).[206] However, some areas of the brain are shown to be immune to pathological lesions on a regular basis. The tectum of the midbrain, the pontine nucleus, the inferior olive, the dentate nucleus and the cerebellar white matter are all unchanged. Other areas of the brain with pathological alterations include the frontal lobe, superior frontal gyrus, temporal cortex, cingulate cortex, thalamus and red nucleus.[206] With this in mind, it was decided to look for the histo-architectural changes in the hippocampus, prefrontal cortex of the cerebrum, corpus striatum [basal nuclei] and hypothalamus in the current study with the purpose of objective and consistent documentation of the histological changes in the rodent brains after the administration of the test drugs (captopril, perindopril and losartan).

Rationale for the Immunohistochemical study in PD

There are immunohistochemical methods advocated for the demonstration of Lewy bodies and tau proteins. Immunohistochemistry using antibodies against α-synuclein had shown to be better sensitive method in detecting Lewy body in comparison to histochemical methods and anti-ubiquitin histochemistry.[212] For the demonstration of neuropil elements such as fibres and dots, use of proteinase K as an epitope retrieval method had shown to be superior to many other methods of quantifying the disease process in PD.[213]

There are many reports mapping α-synuclein in the rat brain predicting and quantifying the disease process in rodent model of PD.[214, 215] However, for the want of resources in the present study, immunohistochemistry of Bcl-2 was selected. Bcl-2 is an apoptosis regulator protein. It prevents apoptosis through antioxidant pathways by protecting the integrity of mitochondrial oxidative phosphorylation and limiting the mitochondrial dysfunction.[216]

Expression and overexpression of Bcl-2 protects the neurons from the induced cell death.[217,218] Of late, there are studies demonstrating the expression of Bcl-2 in normal rodent brain. In the normal rodent brain, the limbic system has more Bcl-2. This emphasizes the neuronal self-regulatory mechanisms to protect these neurons from the apoptosis. Its expression had been recorded in some areas of the cerebellar cortex and the hippocampus.[218] This justifies the selection and evaluation of Bcl-2 as marker of neuronal protection from the programmed cell death possibly involving oxidative stress injury in the rodent brains in this study [hippocampus, prefrontal cortex, corpus striatum and hypothalamus] .

Novelty of the study

In this study, the neuroprotective role of two ACE inhibitors, captopril and perindopril were evaluated. Along with these drugs, a novel ARB, losartan was also had been evaluated. For the purpose of this, three animal models [Rotenone, MPTP, Paraquat] were chosen. In order to ascertain a clear neuroprotective environment, it was prudent to evaluate the anti-oxidant, neurotransmitter & inflammatory marker role of these drugs in the same animal models.

There are similar studies in the past concentrating on the beneficial roles of single drug in one of the animal models. This is among few studies that have considered three drugs at a time in three different animal models. Use of multiple

drugs from the same group and / or similar group alleviates the small changes in the drug specific pharmacokinetics and enhances the group specific potency and the efficacy. No animal model is a gold standard in for the reproduction of clinically relevant parkinsonism. Therefore, the use of multiple animal models results in more generalizable outcomes in terms of neuronal loss and clinical spectrum of manifestations.

In the present study, five different outcomes are clubbed [Neuobehavioral analysis, Assay of oxidative stress, Estimation of neurotransmitter and inflammatory marker, histopathological evaluation and immunohistochemistry study]. Neurobehavioural analysis measures not only the motor manifestations, but also anxiety, depression and muscle strength. In addition to the anti-oxidative enzymes level estimation, neurotransmitters level and inflammatory marker level assay has added a second dimension for the study outcomes that had focused on the neuroprotective role of these drugs in the animal model. This aspect is gaining importance in the pathogenesis and progression of PD. Most importantly, this study also evaluated the possible beneficial role of drugs affecting the brain angiotensin system, it was imperative to study the histo-architectural changes in the various parts of brain [hippocampus, prefrontal cortex, corpus striatum & hypothalamus] rodent model, and to ascertain the outcomes with the documentation, immunohistochemistry study with the anti-apoptotic marker [Bcl-2] had also been done.

To our knowledge, this was the first robust animal model study in which three drugs were evaluated across five study outcomes (neurobehavioral, oxidative stress markers, neurotransmitters and inflammatory marker, histopathological changes - H & E, and immunohistochemistry) in three animal models [Rotenone, MPTP and Paraquat models].

Therefore, the study outcomes of our study are more generalizable in rodent model. As the outcome evaluates all the aspects of PD from the neuronal loss to clinical manifestations, to the possible pathological processes of causation – this study arrived at more holistic and realistic results.

MATERIALS & METHODS

MATERIALS & METHODS

Animal Ethical Clearance:

The Institutional Animal Ethical Committee of BLDEU's Shri B M Patil Medical College in Vijayapura, Karnataka state (registered with CPCSEA, India) accepted the study protocol (No. 33/16 on January 16, 2016) prior to the start of the study. The CPCSEA guidelines were followed throughout the study.

Study design:

Experimental In-Vivo and In-Vitro study in wistar albino rats and swiss albino mice.

The data obtained from the animal studies give more valuable input from the preclinical studies further to have the similar studies in the human in larger scale (clinical trials), before the drug is being used for the same indication. Ultimately, the animal studies would provide more insight for further similar studies in humans. Easy availability, easy handling, high reproducibility and similar to human physiology made rat and mice to be used for the current study.

Approximate total duration of the study: 36 months

Number of groups to be studied: 17 with each group having 6 animals

Total sample size of the study: Wistar Albino rats = 36; Swiss Albino mice = 66; **Total:** 102

Scientific basis of sample size used in the study:

Minimum number of animals in each group is 6 to draw the valid statistical conclusion.[23,219]

Sampling technique used in the study: Judgemental sampling

Experimental animals

Detailed description of the groups:

The neuroprotective role of drugs that modify renin angiotensin system on histoanatomical structures of brain in animal models of PD studied in wistar albino rats and swiss albino mice. The detailed description of study groups were as follows:[17-22]

Table 6: Description of the study groups

Model I: Rotenone Model in wistar albino rats	
Group I	***Vehicle control*** for Rotenone model [Equivalent normal saline i.p]
Group II	Rotenone [3 mg/kg BW i.p daily for 21 days]; [***Negative control***]
Group III	[Levodopa 12 mg/kg and Benserazide 3 mg/kg BW i.p daily for 25 days] + [Rotenone 3 mg/kg BW i.p daily for 21 days] [***Positive control***]
Group IV	**Captopril** 20 mg/kg BW i.p daily for 25 days + Rotenone [3 mg/kg BW i.p daily for 21 days]
Group V	**Perindopril** 5 mg/kg BW i.p daily for 25 days + Rotenone [3 mg/kg BW i.p daily for 21 days]
Group VI	**Losartan** 90 mg/kg BW i.p daily for 25 days + Rotenone [3 mg/kg BW i.p daily for 21 days]
Model II: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP] Model in swiss albino mice	
Group I	***Vehicle control*** for MPTP model [Equivalent normal saline i.p]
Group II	MPTP [25 mg/kg BW s.c]; Total 10 doses at an interval of 3.5 days [***Negative control***]
Group III	[Levodopa 12 mg/kg and Benserazide 3 mg/kg BW i.p daily for 35 days] + [MPTP 25 mg/kg BW s.c; Total 10 doses at an interval of 3.5 days] [***Positive control***]
Group IV	**Captopril** 20 mg/kg BW i.p daily for 35 days + MPTP [25 mg/kg BW i.p ; Total 10 doses at an interval of 3.5 days]
Group V	**Perindopril** 5 mg/kg BW i.p daily for 35 days + MPTP [25 mg/kg BW i.p ; Total 10 doses at an interval of 3.5 days]
Group VI	**Losartan** 90 mg/kg BW i.p daily for 35 days + MPTP [25 mg/kg BW i.p ; Total 10 doses at an interval of 3.5 days]
Model III: Paraquat Model in swiss albino mice	
Group I	***Vehicle control*** for Paraquat model *[Same Group 1 will be considered as Vehicle Control in this Model also] [Equivalent normal saline i.p]*
Group II	Paraquat [7 mg/kg BW i.p]; Total 10 doses at an interval of 2 days [***Negative control***]
Group III	[Levodopa 12 mg/kg and Benserazide 3 mg/kg BW i.p daily for 25 days] + [Paraquat 7 mg/kg BW i.p; Total 10 doses at an interval of 2 days] [***Positive control***]
Group IV	**Captopril** 20 mg/kg BW i.p daily for 25 days + Paraquat [7 mg/kg BW i.p; Total 10 doses at an interval of 2 days]
Group V	**Perindopril** 5 mg/kg BW i.p daily for 25 days + Paraquat [7 mg/kg BW i.p; Total 10 doses at an interval of 2 days]
Group VI	**Losartan** 90 mg/kg BW i.p daily for 25 days + Paraquat [7 mg/kg BW i.p; Total 10 doses at an interval of 2 days]

* **MPTP** -1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine**; BW**-Body weight; **s.c** Subcutaneous; **i.p**-Intraperitoneal.

Study setting and animals

Procedure in detail:

Healthy adult wistar albino rats of either sex weighing 180–250 gm were chosen for the Rotenone. Healthy adult Swiss albino mice of either sex weighing 20–30 gm were chosen for the MPTP and paraquat studies. All of the animals were procured from the BLDEU's Shri B M Patil Medical College's Animal House in Vijayapura, Karnataka.

The guidelines of Animal Good Laboratory Practices [GLP] and the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA] [Indian standard guidelines] were followed during the research. Throughout the trial, the animals were kept in spacious, airy and hygienic cages. The animals had a 12-hour day and night schedule with a temperature of [64-79°F] [29] kept at standard experimental conditions. Rodents were allowed to acclimate in the animal house under normal settings. Throughout the study, the animals were provided with a commercial pellet diet and water ad libitum. The animals were fasted for 12 hours before the experiment and had only access to water.

Figure 11: Grouping & caging of the rodents [free access to food & water] in the central animal house

Inclusion criteria:

The Rotenone model used healthy** male or female wistar albino rats who were adults (3 years old) and weighed 180–250 gm. For the MPTP and Paraquat models, healthy male or female swiss albino adult mice (2 years old) weighing 20–30 gm were used.

** The following factors were considered in determining whether the animal was healthy or not:

i. Wistar albino rats and swiss albino mice were bred under strict supervision in the central animal house following all the guidelines laid by the CPCSEA [National regulatory agency related to usage of laboratory animals for the experiments and research]

ii. The following animal behaviours were observed:

- Feeding habits
- Social interaction
- Physical activity [Locomotor activity]

iii. The weight of the animals were measured.

Exclusion criteria:

Rats and mice with insufficient induction of parkinsonism as judged by examination were excluded. All the dead animals during the induction of PD and during the experimental duration were excluded from the analysis. Unhealthy [diseased], age < 3 years & > 3½ years for the rats and age< 2 years & > 2½ years for the mice and weight < 180 gm & > 250 gm for the rats and weight < 20 gm & > 30 gm for the mice were excluded.

Drugs used in the study:

Details of the drugs used in the study is described as follows:

Table 7: Details of the test drugs used in the study[11-26,23,24]

Sl. No.	Generic Name	Swiss albino mice	Wistar albino rats	Route of administration
1.	Captopril	20 mg	20 mg	i.p
2.	Perindopril	5 mg	5 mg	i.p
3.	Losartan	90 mg	90 mg	i.p

*i.p [Intraperitoneal]

Table no. 8: Details of experimental and standard drugs used in the study[17-24]

Sl.No.	Drug		Company Pvt. Ltd. [India]/Abroad	Formulation	Dose [mg/kg]	ROA
	Generic Name	Brand Name				
1.	Rotenone	Not applicable	Sigma-Aldrich	Powder	3 mg/kg/day for 21 days in wistar albino rats	i.p
2.	MPTP [1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine]	Not applicable	Sigma-Aldrich	Powder	25 mg/kg; Total 10 doses at an interval of 3.5 days in swiss albino mice	i.p
3.	Paraquat	Not applicable	Sigma-Aldrich	Powder	7 mg/kg; Total 10 doses at an interval of 2 days in swiss albino mice	i.p
4.	Levodopa + Benserazide	Not applicable	Sigma-Aldrich	Tablet	12 + 3#	i.p
5.	Ketamine	Ketam	Sun Pharma	Injection	80	i.p
6.	Carboxymethyl cellulose	Not applicable	Loba chemie	Powder	Not applicable	

*** i.p:** Intraperitoneal; **ROA**: Route of administration; #Levodopa 12 mg/kg + Benserazide 3 mg/kg body weight of animals.

Vehicle control groups for Rotenone, MPTP and Paraquat models

Six healthy adult wistar albino rats were grouped and labelled as "vehicle control" group (Group I) in the Rotenone model. Normal saline was given intraperitoneally to these rats concurrently experimental groups received their drugs. The vehicle control and experimental groups underwent the same behavioural analysis, anti-oxidant assay, neurotransmitter & inflammatory marker estimation, brain histopathological evaluation and immunohistochemistry study. In MPTP and paraquat, six healthy adult swiss albino mice were grouped and labelled as vehicle control group (Group I). Normal saline was injected intraperitoneally into these animals at the same time that the drugs were given to the experimental groups. To decrease the number of animals in the study, the same control group [Group I] was used in both the MPTP and paraquat models. Each group was tested for behavioural analysis, anti-oxidant assay, neurotransmitter & inflammatory marker estimation, brain tissue histopathological evaluation and immunohistochemistry study.

Rotenone model

The Rotenone solution (Sigma Chemicals, Mumbai) was freshly formulated at a concentration of 3 mg/kg. Rotenone was dissolved in dimethyl sulfoxide, and potassium hydroxide was used to adjust the pH to 7.4. Rotenone was administered i.p at a dose of 3 mg/kg body weight for seven days. Because the solution was only stable for 24 hours at 25°C, it was utilised immediately after preparation.

Table 9: Details of groups in rotenone model

Group	Group specification
Group I	Vehicle control: Equivalent normal saline (i.p)
Group II	Negative control: Rotenone (3 mg/kg BW i.p)
Group III	Positive control: Levodopa (12 mg/Kg) and Benserazide (3 mg/kg BW i.p) + Rotenone (3 mg/kg BW i.p)
Group IV	Captopril (20mg/kg BW i.p) + Rotenone (3 mg/kg BW i.p)
Group V	Perindopril (5mg/kg BW i.p) + Rotenone (3 mg/kg BW i.p)
Group VI	Losartan (90 mg/Kg BW i.p) + Rotenone (3 mg/kg BW i.p)

Figure 12: Rotenone preparation used in the study

Methyl phenyl tetrahydropyridine (MPTP) model

To avoid decomposition, the MPTP (Sigma Chemicals, Mumbai) was stored at 37°C according to the manufacturer's instructions. The MPTP solution was freshly produced at a concentration of 25 mg/kg. The MPTP was dissolved in a 0.90 percent sodium chloride solution. It was given intraperitoneally at a dose of 25 mg/kg body weight for seven days. As the MPTP solution is only stable for 24 hours at 40°C, it was used right away after preparation.

Table 10: Details of groups used in the MPTP model

Group	Group specification
Group I	Vehicle control: Equivalent normal saline (i.p) (same group was used in Paraquat model also to reduce the number of animals in the study)
Group II	Negative control: MPTP (25 mg/kg BW i.p)
Group III	Positive control: Levodopa (12 mg/kg) and Benserazide (3 mg/kg BW i.p) + MPTP (25 mg/kg BW i.p)
Group IV	Captopril (20 mg/kg BW i.p) + MPTP (25 mg/kg BW i.p)
Group V	Perindopril (5 mg/kg BW i.p) + MPTP (25 mg/kg BW i.p)
Group VI	Losartan (90 mg/kg BW i.p) + MPTP (25 mg/kg BW i.p)

Figure 13: MPTP preparation used in the study

Paraquat model

The paraquat (Sigma chemicals, Mumbai) was stored according to the manufacturer's instructions to avoid decomposition. The paraquat solution was made fresh at a concentration of 7 mg per kg of body weight. At a two-day interval, 7mg/kg of body weight of paraquat was injected intraperitoneally.

Table 11: Details of groups used in the Paraquat model

Group	Group specification
Group I	Vehicle control group - Equivalent normal saline (i.p) (same group is used in MPTP model also to reduce the number of animals in the study)
Group II	Negative control: Paraquat (25 mg/kg BW i.p)
Group III	Positive control: Levodopa (12 mg/kg) and Benserazide (3 mg/kg BW i.p) + paraquat (7 mg/kg BW i.p)
Group IV	Captopril (20mg/kg BW i.p) + Paraquat (7 mg/kg BW i.p)
Group V	Perindopril (5mg/kg BW i.p) + Paraquat (7 mg/kg BW i.p)
Group VI	Losartan (90 mg/kg BW i.p) + Paraquat (7 mg/kg BW i.p)

Figure 14: Paraquat preparation used in the study

Tremors, bradykinesia, postural instability, gait disturbances, and rigidity were all assessed twice daily using a checklist for the presence of these parkinsonian symptoms in rodents.

Figure 15: Test drug preparations that were used to give intraperitoneal injections

Figure 16: Intraperitoneal injection in the animal models to various groups

The study did not include animals that died after receiving the intraperitoneal injection. Animals that did not show any signs of PD were likewise ruled out. Animals that died within minutes of receiving an injection, and the animals which did not show any signs of PD were also not included in the study. All animals that could

not survive intraperitoneal injections for seven days were also removed from the study.

Behavioural analysis

Spontaneous locomotor activity, the rota rod test and the grip strength test were used to assess motor functioning. The hole board test was used to assess exploratory behaviour. The effects of depression on behaviour were explored using a forced swim test and a tail suspension test. The elevated plus maze test was used to examine how anxiety affects behaviour.

Spontaneous locomotor activity:

The actophotometer, which works with photoelectric cells connected with a counter, was used to monitor spontaneous horizontal activity. During the testing period, the device was put in a sound-attenuated and ventilated room. Before beginning the real locomotor activity task for the next 3 minutes, all of the rats/mice were individually placed in the activity cage for 3 minutes to habituate them. The baseline activity score was taken into consideration. The activity counts were counted in arbitrary units based on the beam breaks generated by the animal's movement. The locomotor activity was measured in counts per 10 minutes.[220]

Figure 17: Measurement of locomotor activity using actophotometer [Total number of counts in 10 minutes]

Rotarod test:

The rodents were placed on a horizontally mounted revolving rod to conduct a motor coordination test. A week before the drug was administered, the animals were trained for three minutes at 25 rpm in the trial. A 5 minutes rest period was offered after each experiment to relieve stress and fatigue. Motor coordination was assessed by comparing the latency to fall on the very first test between the treatment groups. The time taken by the animals to fall from the rotating rod was recorded. The length of time (duration) that the animal remained on the rod without falling was used to determine their coordination, balance, physical condition and motor-planning abilities. A cut off period of 240 seconds (4 minutes) was fixed, and each animal was tested three times at a 10 minutes interval.[221]

Figure 18: Measurement of motor co-ordination using rotarod apparatus [Fall of time in seconds]

Grip strength test:

Each animal's grip strength was tested by suspending the animal on a thread [at a height of 50 cm] with their front paws and recordings were taken in seconds.[23,25,26]

Hole board test:

Rats and mice were placed on a 25 cm elevated wooden board with 16 holes (40 cm x 40 cm). Each hole had a diameter of 3 cm and was spaced at regular intervals. Animals were placed on the corner of the apparatus and counted how many times their heads dipped for the next 5 minutes. When the animal dipped its head into any hole in the box up to the level of the ears, it was counted as a head dipping. Between each subject, the apparatus was carefully cleaned. Increased exploration of the holes was associated with a decrease in anxiety.[222]

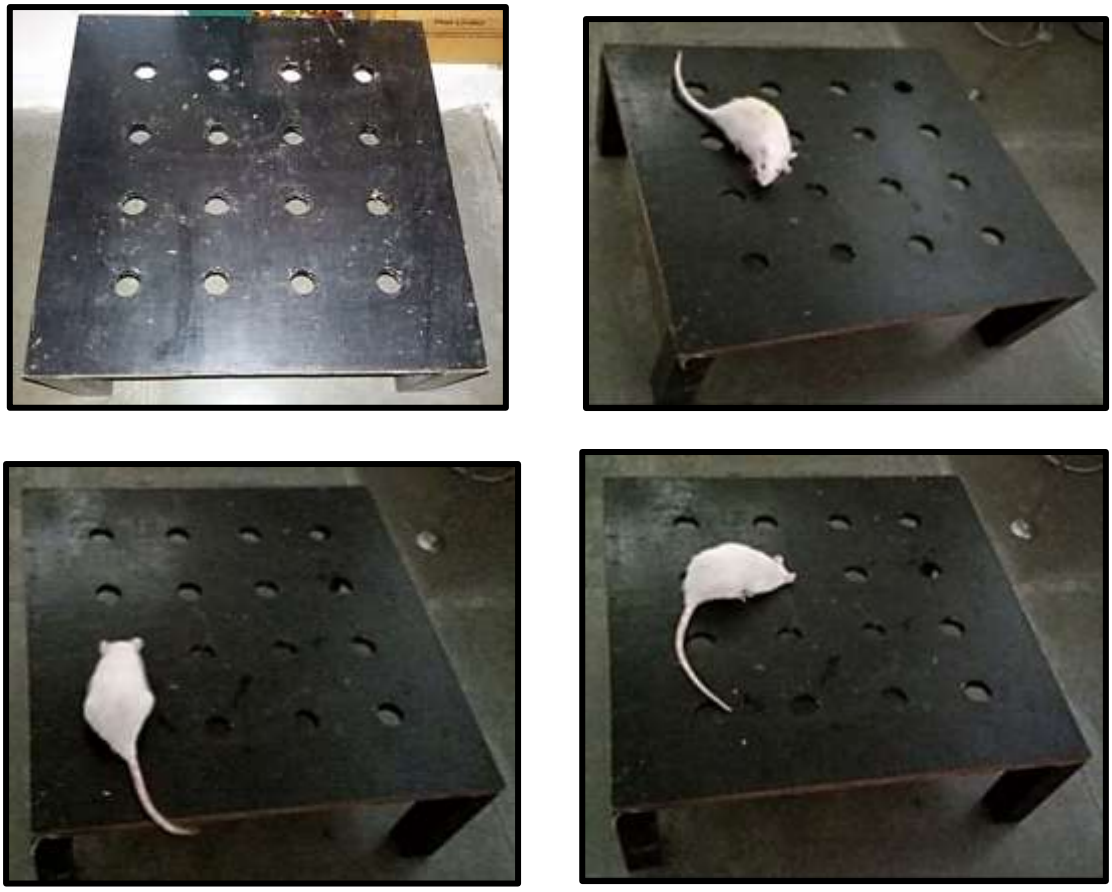


Figure 19: Measurement of exploratory behaviour by hole board test [Number of nose pickings in 5 minutes]

Tail suspension test:

The rats'/mice's tails were carefully lifted and held in place on a support. The grid had been reversed, causing creatures to dangle from it upside down. To save the

animal from falling and being injured, the grid was only 20 cm above the ground level. A three-inch wall was added to the grid to prevent animals from progressing to the higher levels. The animals had to stay on the grid for a total of 240 seconds (4 minutes).[223] The tail-hanging time was calculated over ten trials, with a one-minute break between them. The immobility period was defined as the amount of time the animal was passively hanging. This was a measure of depression.

Figure 20: Evaluation of depression behaviour with tail suspension test [Immobility time in seconds in total 5 minutes test]

Forced swim test:

Rats/mice were placed in an open cylinder with a diameter of 10 cm and a height of 25 cm, filled to a height of 15 cm with water at a temperature of 25°C. In this limited space, the animals were forced to swim. As a result, there was a period of immobility. When the animal quit struggling to get out and settled into a state of floating motionless with just a few small movements to keep its head above water, the time was recorded. The total duration of the FST was set to 240 seconds (4 minutes). Each animal was videotaped in its totality and then analysed on a computer. The duration of each animal's motion was assessed and recorded during this behavioural

analysis. From 240 seconds, the entire mobility time was removed. This signified the period of immobility. This method was chosen because it was preferable to observe the movements rather than the lack of movements. Mobility or movement was defined as any movement other than those required to maintain bodily balance and keep the head above water.[224]

Figure 21: Evaluation of depression with forced swim test [Immobility time in seconds in last 4 minutes [of total 6 minutes test]

Elevated plus maze test:

The plus maze for rats and mice had two perpendicular open arms (30 x 5 cm) and two closed arms (30 x 5 x 15 cm) that were equally perpendicular. The animals were placed with four arms facing an open arm at the maze's intersection. A video-tracking system and an observer concurrently recorded the duration of each arm. After placing the animal in the centre, the movement of the animal was recorded for 300 seconds (5 minutes). When the animal entered the open arm with all four paws, the number of open arm entries was counted. Complete entry was defined as one paw completely leaving the arm or intersection. The amount of time it spent in the open arm was called open arm time. The number of entries and time spent in the closed arm were also kept track of. The percentage of time spent by the rats/mice in the open arm

was used as an indicator of anxiety-like behaviour. The time spent in open arms was divided by the total observed time to get the percentage of time spent in open arms (TOA percent) (300 seconds). The percentage of entries into the open arm was calculated by dividing the total number of entries into the closed and open arms by the number of entries into the open arm (EOA percent).[23,24]

First arm [Open/Closed] preference

- Number of entries into the open and closed arms
- Time [in seconds] spent in open arm and closed arms

Figure 22: Evaluation of anxiety behaviour through elevated plus maze test

Dissection of brain and processing of the two hemispheres

All the rats and mice were anaesthetized with thiopental sodium (50 mg/kg) after 24 hours of analysis. Cervical decapitation was used to sacrifice all of the rats and mice. The cranial cavity was opened and the brain was dissected out. The right and left halves of each brain were hemisectioned along the longitudinal fissure. The oxidative stress markers,

neurotransmitters and inflammatory marker were measured in one hemisection after homogenisation. The other hemisection was formalin-fixed and used for histological and immunohistochemical analysis.

Disposal Method:

The carcass was disposed of according to the Indian standard guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA].

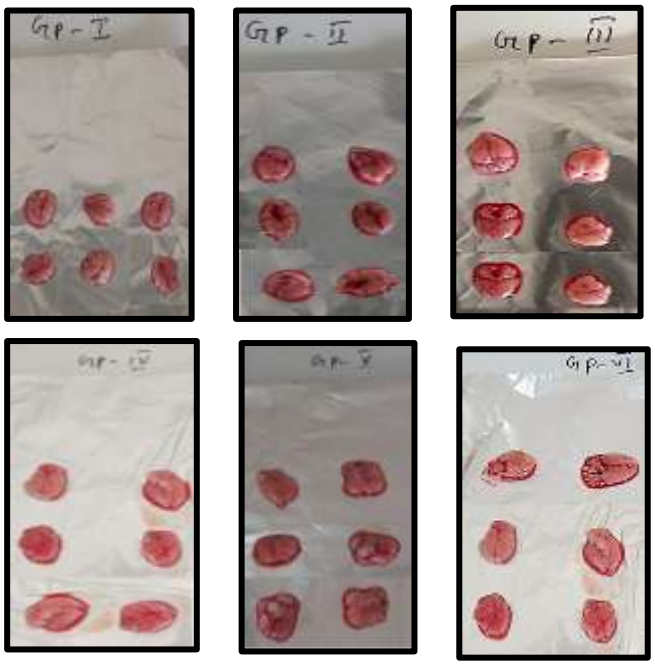


Figure 23: Dissected brain specimens from 6 groups in a model

Estimation of brain antioxidants, neurotransmitters and inflammatory markers

A homogenizer was used to homogenise the hemisections of brain tissue dissected, for 1 minute in 5 mL of HCl-butanol. Centrifugation at 2000 rpm for 10 minutes was used to remove the unbroken cells. A portion of the supernatant phase (1 mL) was taken and placed in a centrifuge tube with 2.5 mL of heptane and 0.31 mL of 0.1 M HCL. After 10 minutes of vigorous shaking, the tube was centrifuged under the same conditions as before to separate the two phases, and the overlaying organic

phase was discarded.[225] The aqueous phase (0.2 ml) was taken for the assay of antioxidants [SOD, Glutathione peroxidase, Reduced glutathione, Catalase and Lipid peroxidation], neurotransmitters [Serotonin (5-HT), Dopamine (DA), Gamma-amino butyric acid (GABA), Glutamate and Acetylcholine (ACh) and inflammatory marker [Myeloperoxidase (MPO)]. All of the steps were completed at 0°C.

Estimation of total protein: The 0.1 mL of homogenate was mixed with 0.9 mL of water and 4.5 mL of alkaline copper sulphate reagent at room temperature for 10 minutes. Folin's reagent (0.5 mL) was added to this. The colour obtained after 20 minutes was measured at 640 nm. The amount of protein in each tissue was measured in mg/g/tissue.[226]

Estimation of Oxidative Stress Markers

- Superoxide Dismutase (SOD)
- Glutathione Peroxidase (GPx)
- Reduced Glutathione (GSH)
- Catalase (CAT)
- Lipid Peroxidasation (LPO)

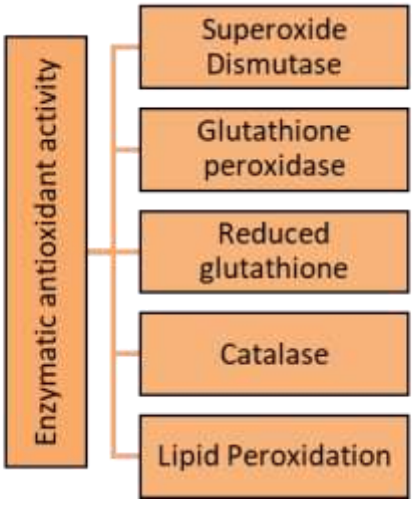


Figure 24: Schematic representation of oxidative stress markers selected for the study

Superoxide Dismutase (SOD) Level: 0.1mL of homogenate supernatant, 0.1mL Ethylene-diamine-tetra-acetic acid, EDTA (1×10^{-4} M), 0.5mL of carbonate buffer, and 1mL of epinephrine (1mM) were mixed together. The combination was spectrophotometrically measured for 3 minutes at 480nm. The activity of SOD was measured in units of U/min/mg.[227]

Estimation glutathione peroxidase: A 3-ml cuvette was filled with 2.0mL of phosphate buffer (75mmol/L, pH 7.0), 50mL of glutathione reductase solution, 50µL (0.12mol/L) NaN3, 0.1mL(0.15mol/L) Na2 EDTA, 100mL(3.0mmol/L) NADPH, and 100mL of tissue supernatant was added. To make a total volume of 2.9 ml, water was added. The reaction was begun by adding 100L of (7.5mmol/L) H_20_2, and the conversion of NADPH to NADP was measured by using a UV spectrophotometer to continuously record the change in absorbance at 340nm at 1 min intervals for 5 minutes. The enzyme activity of GPx was measured in mg of proteins.[227]

Estimation of reduced glutathione: 1mL of 5% TCA was added to 250µL of tissue homogenate in a 2 mL eppendroff tube, and the mixture was centrifuged at 3000rpm for 10 minutes at room temperature. 1.5ml of 0.2M phosphate buffer was added to 250µL of the aforesaid supernatant and thoroughly mixed. The absorbance was measured at 412nm within 10 minutes after adding 250µL of 0.6mM Ellman's reagent (DTNB solution) to the above mixture. The glutathione reduction solution (1 mg/mL) was used to create a standard graph, and the GSH level in the tissue homogenates was estimated using interpolation. Glutathione concentration is measured as µg/mg of protein.[228]

Catalase activity: 1.95mL of 50nM phosphate buffer and 1mL of 30mM hydrogen peroxide were added to the homogenate mixture. At 15 seconds intervals, the catalase

activity was measured at 240nm. The catalase activity was calculated using the change in catalase absorbance/minute as a result of the hydrogen peroxide extinction coefficient (0.071mmol cm-1). Catalase activity was measured in micromoles of H_2O_2 oxidised per milligrams of protein per minute.[227]

Lipid peroxidation assay: To 100L of tissue homogenate, 2mL of (1:1:1) thiobarbituric acid reagent (thiobarbituric acid 0.37 %, 0.25N hydrochloric acid, and 15% trichloroacetic acid) was added and mixed. The above-mentioned substance was heated for 15 minutes in a boiling water bath, then cooled and centrifuged at room temperature for 10 minutes at 3500 rpm. The pink colour developed was quantified at 535nm in a UV spectrophotometer against a reagent. LPO was measured in nmol of MDA/mg/of protein.[229]

Estimation of Neurotransmitter Levels

- Serotonin (5-HT)
- Dopamine (DA)
- GABA
- Glutamate
- Acetylcholine (ACh)

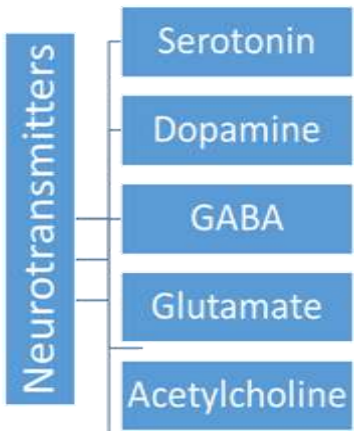


Figure 25: Schematic representation of neurotransmitters assayed in the study

Serotonin assay: To 0.2ml of aqueous phase, 0.25ml of OPT reagent was added. The fluorophore was produced by heating it to 100°C for 10 minutes. In the spectrophotometer, readings for serotonin at 360-470nm was taken after the samples had reached the equilibrium with the ambient temperature.[230]

Dopamine assay: 0.05ml of 0.4 MHCL and 0.1ml of sodium acetate buffer (pH 6.9) were added to the 0.2ml of aqueous phase, followed by 0.1ml of iodine solution (0.1M in ethanol) for oxidation. After 2 minutes, the reaction was halted by adding 0.1mL of Na_2SO_3 solution. After 1.5 minutes, 0.1mL of acetic acid was added. When the sample reached room temperature, the solution was heated to 100°C for 6 minutes, and the spectrophotometer was used to read the absorption and emission spectra. Dopamine was measured at 330-375nm.[231]

GABA assay: A 0.1ml sample of tissue extract was mixed with 0.2ml of 0.14M Ninhydrin solution in 0.5M carbonate-bicarbonate buffer (pH 9.95) and kept in a water bath at 60°C for 30 minutes. After cooling, the samples were treated with 5ml of copper tartarate reagent (0.16% disodium carbonate, 0.03% copper sulphate, and 0.0329% tartaric acid). Fluorescence at 377/455nm was measured in a spectrophotometer after 10 minutes.[232]

Glutamate assay: To a boiling and ice-cooled supernatant extract of ninhydrin mixed brain homogenate, 0.4mL guanidine carbonate was added. 1 ml of 100mM lead acetate, 0.5ml of 1 N NaOH, and 6ml of dH2O were added to this mixture. Under ice-cold conditions, 0.1% 2,4-dinitrophenyl hydrazine dissolved in 0.01N HCl was added to this mixture and incubated for 30 minutes. An UV spectrophotometer was used to measure the colour intensity of this combination at 420nm. The glutamate levels were measured in μg of monoamine/g of wet tissue weight.[233]

ACH assay: To activate acetylcholinesterase, the brain homogenate tissues were boiled. The bound acetylcholine was then released. The addition of ferric chloride solution resulted in the formation of a brown-colored solution. At 540nm, it was measured spectrophotometrically. The acetylcholine content was measured in moles of acetylcholine/g of wet tissue weight.[234]

Estimation of Inflammatory marker

Myeloperoxidase (MPO) activity estimation: The tetra-methyl-benzidine technique was used to measure MPO. A 10µl sample was added to an 80µl 0.75mM H_2O_2 and 110µl TMB solution. For 5 minutes, the mixture was incubated at 37°C. The reaction was halted with 50µl 2M H_2SO_4 and MPO activity was determined by measuring absorbance at 450nm.[235]

Figure 26: Homogeniser used in the study

Figure 27: Centrifuge used in the study

Figure 28: UV Spectrophotometer used in the study

Histopathological evaluation

The histological examination and immunohistochemistry analysis were performed on the other hemisection of each experimental animal [one hemisection had already been used for biochemical analysis]. The microscopical examination of tissues for pathological changes is known as histopathology. The following stages were involved: collection of morbid tissues [hemisection of the brain], fixation, sections preparation, staining and microscopic evaluation.

.

Collection of materials

Thin pieces of 3 to 5 mm, thickness were collected from the tissues showing gross morbid changes along with normal tissue.

Fixation:

Fixation was the first step towards the preparation of a histological section from a dead biological specimen. The substances used for fixation are called as fixatives.

Common Fixatives: Formalin, Zenker's fluids, Bouin's fluid

10% Formalin was used as a fixative and the tissue was kept in fixative for 24-48 hours at room temperature.

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein
b) Prevents autolysis
c) Preserves the structure of the tissue, and
d) Prevents shrinkage

Washing: After fixation tissue is washed under running tap water one to two hours, it removes the fixative from tissue.

Dehydration: It was done by dipping the tissue one time in the following solutions:

30% Alcohol

50% Alcohol

80% Alcohol

80% Alcohol

90% Alcohol

100% Alcohol

100% Alcohol

100% Alcohol

Clearing was done by two dips in Xylene consecutively.

Preparation of sections:

Infiltration: Tissues were infiltrated with paraffin by dipping three times consecutively in paraffin at 50-56^{o}C.

Embedding: Was done by using L-blocks. The tissue was put in it over which the melted paraffin was poured which solidified slowly.

Section cutting: The tissues were sectioned into thin slices by using microtome.

Haematoxylin & Eosin Method of Staining:

Rehydration – Tissues were dipped in the following solutions serially as follows:

- Xylene 2 minutes
- Xylene 2 minutes
- Absolute alcohol 1 minute
- Absolute alcohol 1 minute
- 90% alcohol 2 minutes
- 70% alcohol 2 minutes
- 50% alcohol 2 minutes
- Distilled water 5 minutes
- Haematoxyllin 2 to 5 minutes [with Harris Haematoxyllin]
- Washed well in running tap water for 2-3 minutes
- Removed excess stain by differentiating in acid alcohol (1% HCL in 70% alcohol) for a few seconds. Blue staining of haematoxylin stained section was changed to red by the action of the acid
- Then, it was immediately washed in alkaline tap water for at least 5 minutes to regain the blue colour
- Dipped in 1% aqueous Eosin 1 to 3 minutes and washed of surplus eosin in water

- Dipped in 90% alcohol for 10 to 15 seconds
- Dipped in Absolute alcohol I agitate for 10 to15 seconds
- Dipped in Absolute alcohol II for 30 seconds
- Dipped in Xylene I for 1 to 2 Minutes
- Dipped in Xylene II for 1 to 2 Minutes
- Mounted on D.P.X and kept the slide for drying

Results:

Nuclei – Blue to blue-black

Nucleolus – Dark blue

Cytoplasm – Pink

Collagen fibers - Lighter pink

Erythrocytes and eosinophil granules - Bright orange red

H and E stained slides showing regional changes in hippocampus, pre frontal cortex [cerebrum], corpus striatum [basal ganglia] and hypothalamus were evaluated for histoarchitectural changes.

Bcl-2 immunohistochemistry: Immunohistochemistry was done with 5-μm-thick pre-treated sections which were placed on L-lysine slides. For anti-apoptotic oncoprotein - Bcl-2 retrieval, the slides were immersed in sodium citrate 0.1M. Slides were preheated in a 750W microwave oven for 7 min. The rodent monoclonal antibody to Bcl-2(Bio SB, Bio Sciences For the World, CA 93117, USA) diluted in 1:100 phosphate buffer saline was used in the study. The slides covered with antibody were placed in a solution jar-containing buffer. Slides were covered with peroxidase blocking and was incubated for 10 minutes. Washed with deionized water and buffer periodically. This was followed by washing with buffer solution 3 times. Then, the slides were placed in substrate 3,3-Diaminobenzidine (DAB) solution for ten minutes

and later washed with buffer. Haematoxylin counter stained the slides.[236] All slides were evaluated for Bcl-2 immunohistochemistry, and the pathology expert opinion was obtained. The scoring system used in the present study was adopted from the criteria defined by Tsuyama et al for Bcl-2 quantification in blood dyscrasias especially B-cell related leukaemia.[236]

Statistical analysis

Data obtained from each model were tabulated separately and subjected to statistical analysis. Then, it was presented as tables and graphs.

Significance level decided before starting of study: $P<0.05$ was considered statistically significant

Statistical tests to be used for data analysis: All the data obtained was entered into Microsoft Office Excel 2007. The data was expressed in Median±SE [For the scores] and Mean±SE [For all the other parameters]. For all the continuous parameters, mean ± standard deviation was calculated for each group. The statistical significance among groups of each model [comparison of the data] was carried out by using one way ANOVA followed by Dunnett post hoc test for data for the data with the Gaussian or normal distribution, and Kruskal-Wallis test was followed by Dunn's post hoc test for the data with non-Gaussian or non-normal distribution.. All the parameters were compared with control group values.

Software(s) to be used for statistical analysis: All calculations for analyzing the data were done with the software SPSS V 20 32bit.

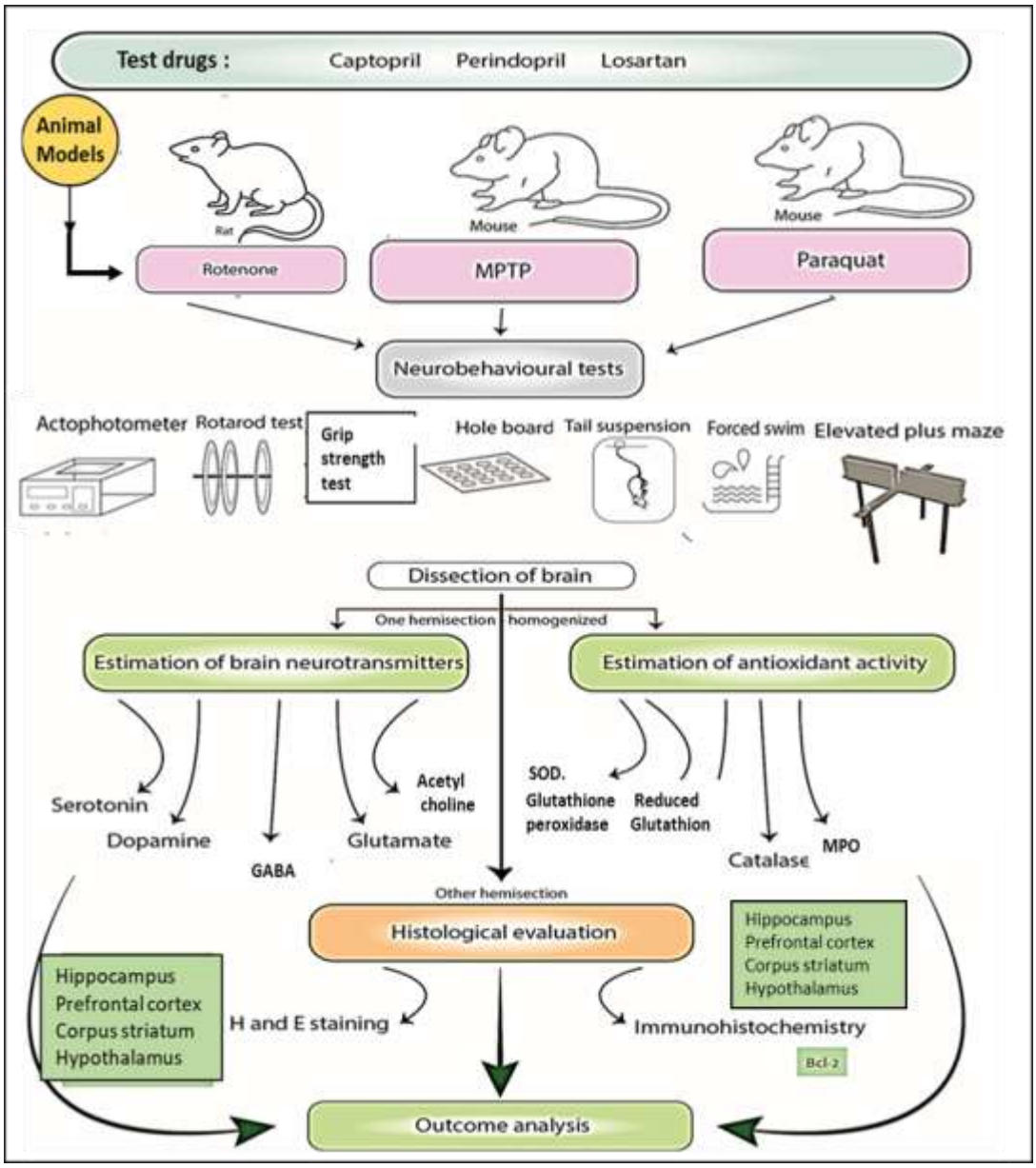


Figure 29: Schematic Representation of Methodology

RESULTS

RESULTS [OBSERVATIONS]

The results (observations) are presented serially as follows:

I. **Rotenone model in wistar albino rats**

II. **MPTP model in swiss albino mice**

III. **Paraquat model in swiss albino mice**

Under the following headings:

- Evaluation of neurobehavioral activity
- Evaluation of oxidative stress markers
- Evaluation of neurotransmitters
- Evaluation of inflammatory marker
- Microanatomy [Histopathological] examination
- Immunohistochemistry study

I. ROTENONE MODEL IN WISTAR ALBINO RATS

Evaluation of neurobehavioral activity

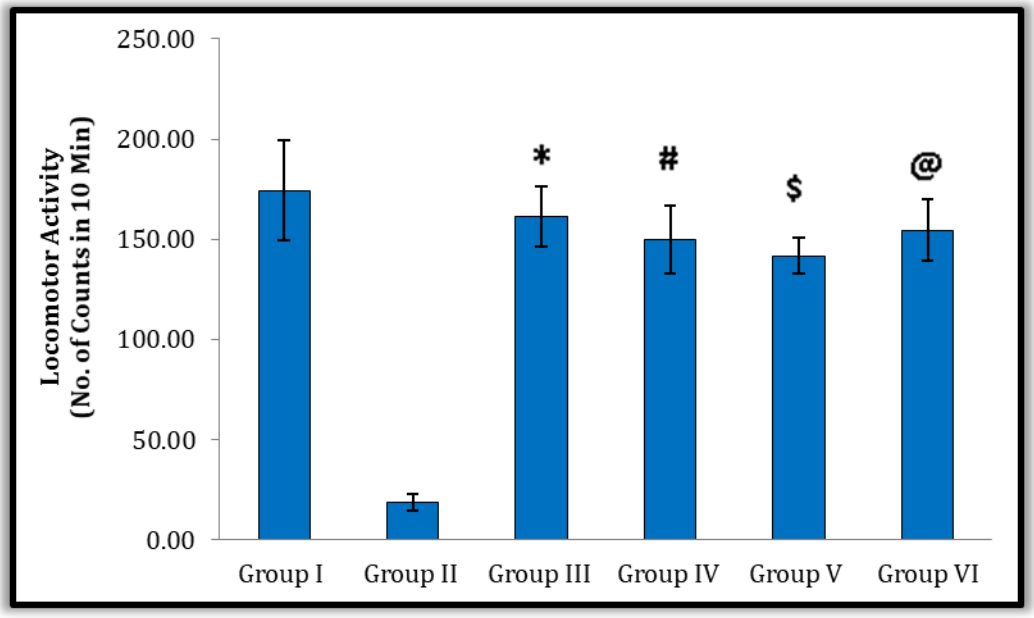


Figure 30: Bar diagram depicting the locomotor activity in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results showed that the standard group [Group III] (Levodopa+Benserazide) had a substantial rise in counts when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group, all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopri and Losartan) showed an increase in the total number of counts, which was statistically significant [Group II] (P<0.05).

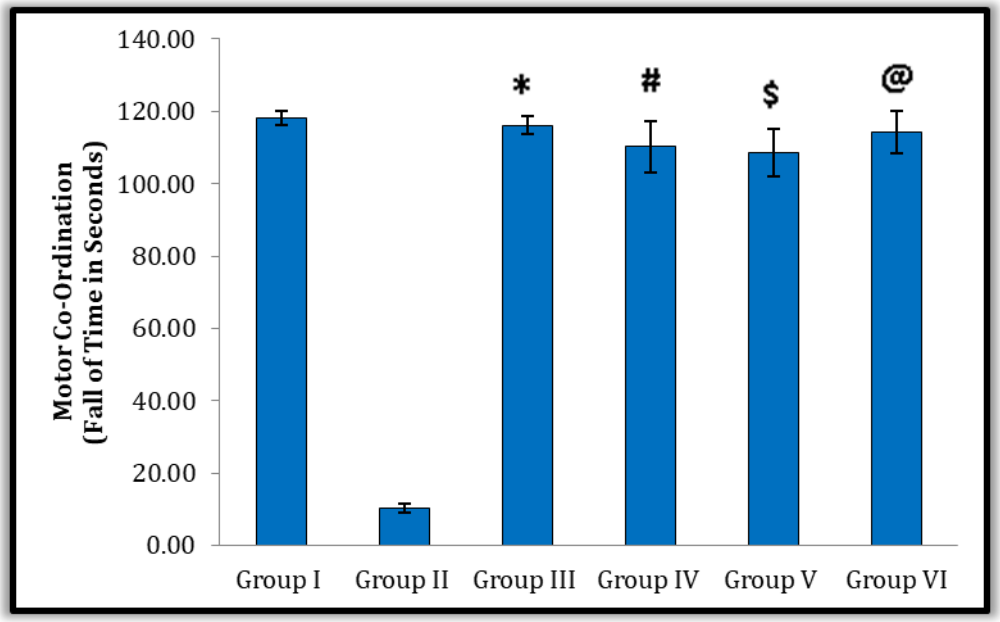


Figure 31: Bar diagram depicting the motor co-ordination in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results showed that the standard group [Group III] (Levodopa+Benserazide) showed a significant increase in the time fall when compared to the negative control group [Group II] (P<0.05). When compared to the

negative control group [Group II], all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) reported an increase in the time fall that was statistically significant (P<0.05).

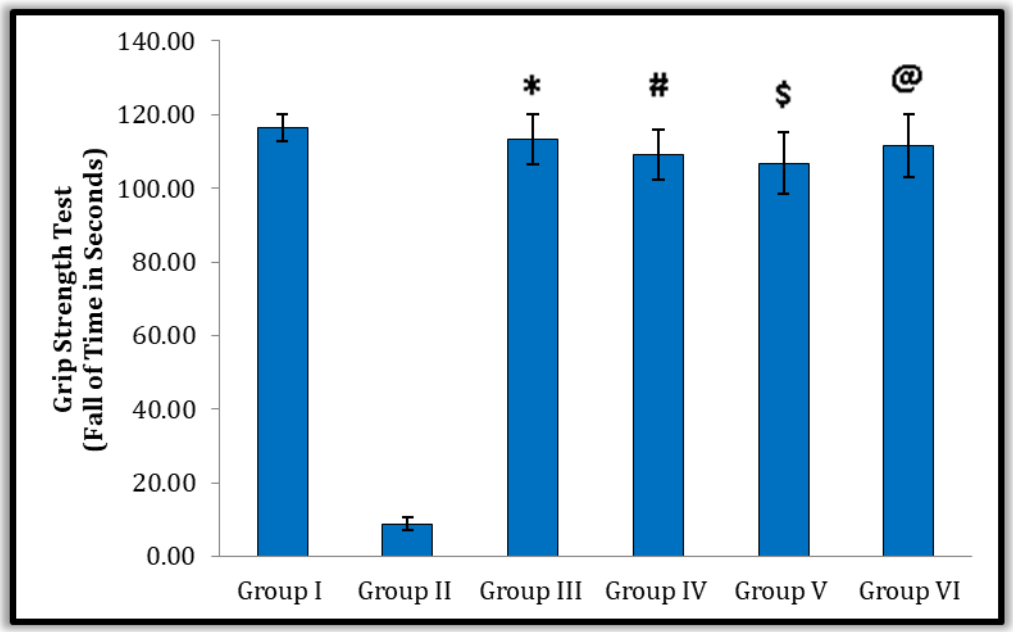


Figure 32: Bar diagram depicting the grip strength test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The Levodopa+Benserazide group (Standard drug group) [Group III] had a significant increase in the parameter (fall of time in Grip Strength Test) as compared to the negative control group [Group II] (P<0.05) When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant (P<0.05) increase in the fall of time.

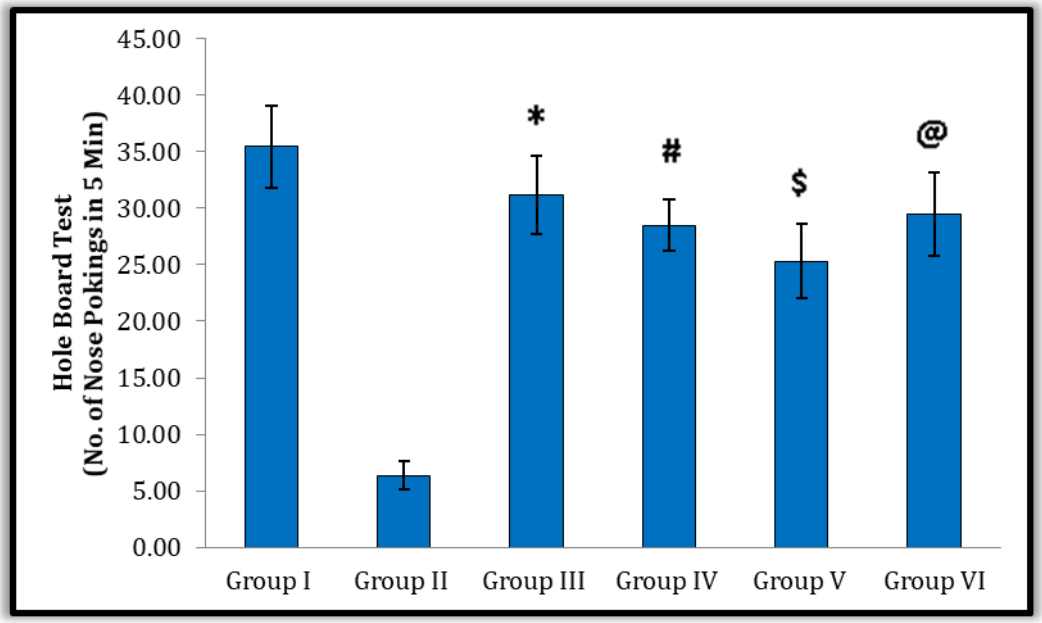


Figure 33: Bar diagram depicting the hole board test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The Levodopa+Benserazide group (Standard drug) [Group III] had a significant increase in the parameter (Nose Poking in Hole Board Test) when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant (P<0.05) increase in nose poking.

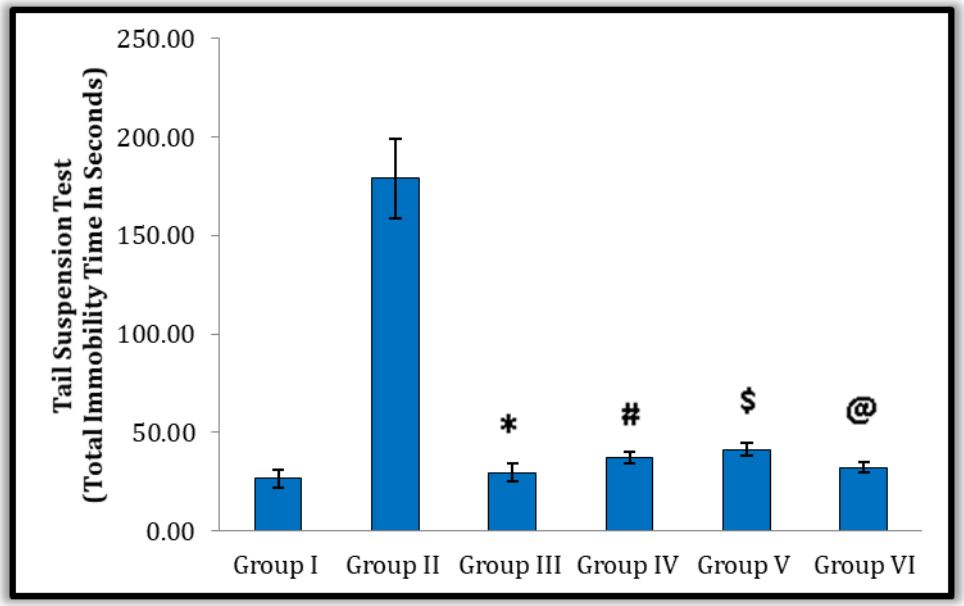


Figure 34: Bar diagram depicting tail suspension test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group (Standard drug group) [Group III] to the negative control group [Group II] (P<0.05), there was a significant decrease in Total Immobility Time in the Tail Suspension Test. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) (Captopril, Perindopril, and Losartan) found a statistically significant (P<0.05) reduction in total immobility time.

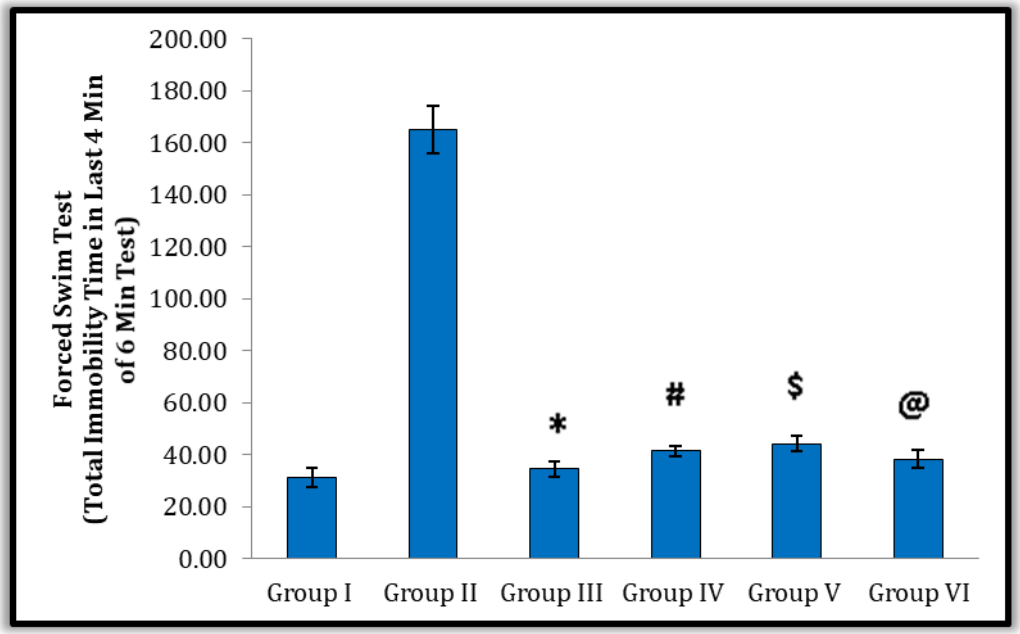


Figure 35: Bar diagram depicting the forced swim test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Similarly, in the parameter (Total Immobility Time in Forced Swim Test), the Levodopa+Benserazide group (Standard drug group) [Group III] showed a significant decrease when compared to the negative control group [Group II](P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a substantial (P<0.05) reduction in total immobility time.

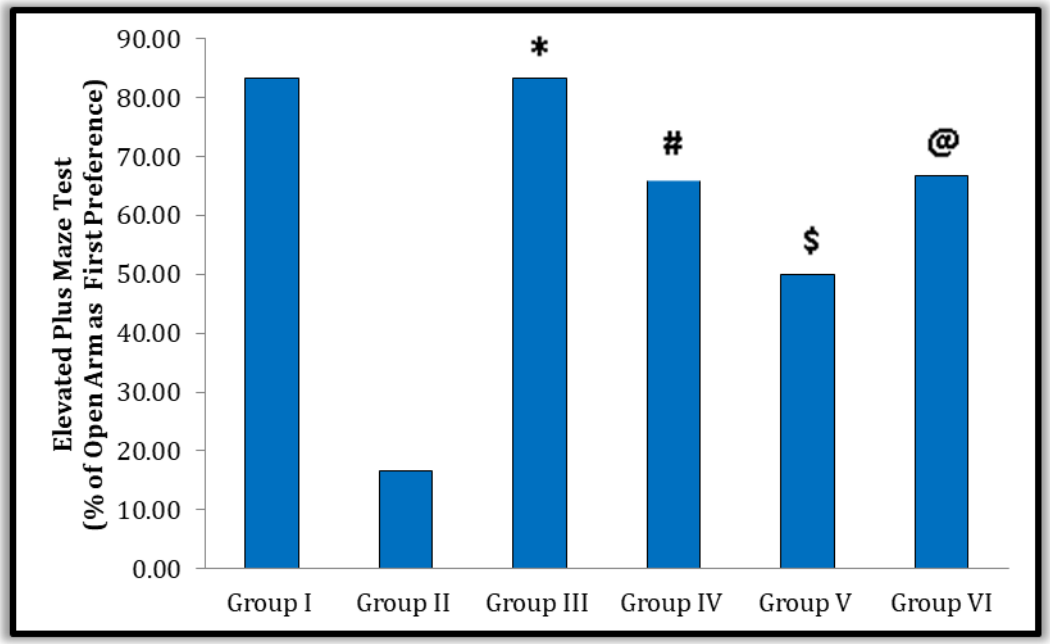


Figure 36: Bar diagram depicting the percentage of open arm as first preference in elevated plus maze test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group (Standard drug group) [Group III] to the negative control group [Group II] in the parameter (% of open arm as first arm preference in the Elevated Plus Maze Test), it was found that there was a significant increase in the Levodopa+Benserazide group (Standard drug group) [Group III] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant (P<0.05) increase in the percentage of open arms as first arm preference.

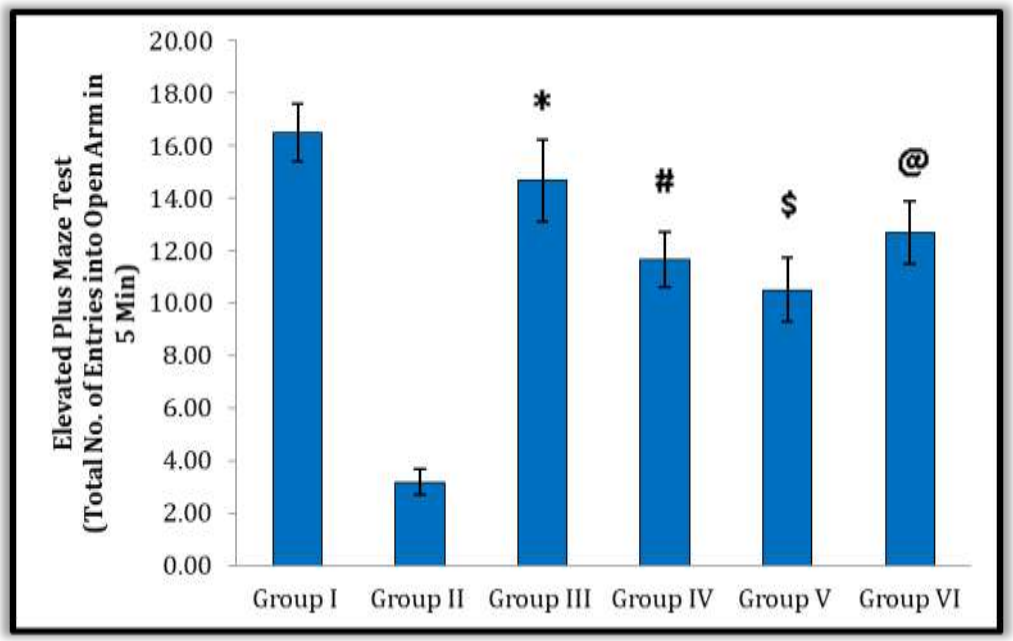


Figure 37: Bar diagram depicting the total number of entries into the open arm in elevated plus maze test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group (Standard drug group) [Group III] to the negative control group [Group II] in the parameter (Total Number of Entries into Open Arm in the Elevated Plus Maze Test), it was found that there was a significant increase in the Levodopa+Benserazide group (Standard drug group) [Group III] ($P<0.05$). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistically significant ($P<0.05$) increase in the total number of entries into the open arm.

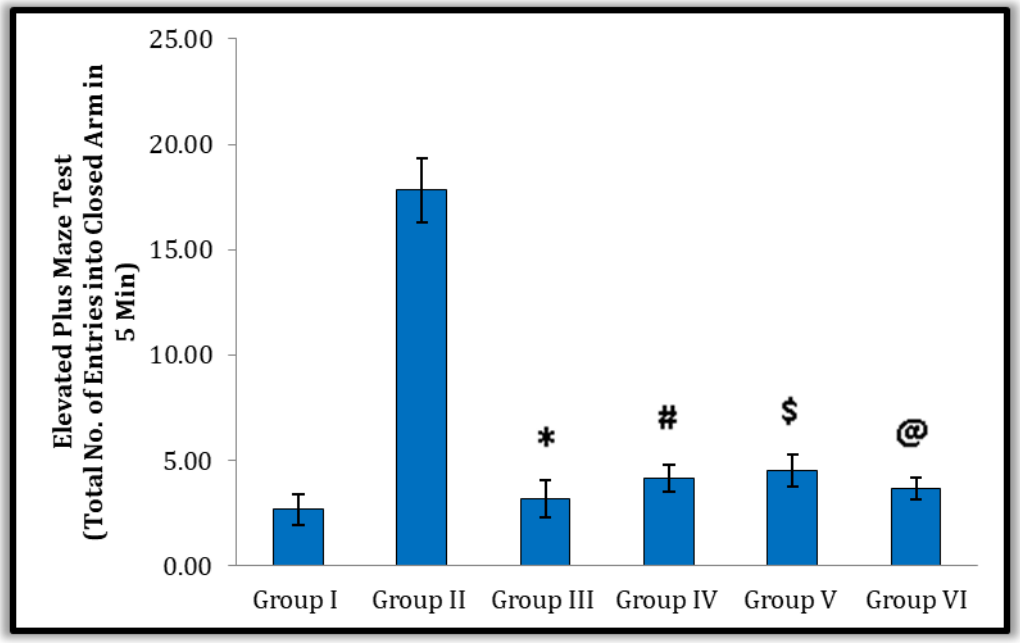


Figure 38: Bar diagram depicting the total number of entries into the closed arm in elevated plus maze test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group (Standard drug group) [Group III] to the negative control group [Group II] in the parameter (Total Number of Entries into Closed Arm in the Elevated Plus Maze Test), it was found that there was a significant decrease in the Levodopa+Benserazide group (Standard drug group) [Group III] (P<0.05). It was also seen that, experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed statistical significant (P<0.05) decrease in the total number of entries into closed arm when compared to the negative control group [Group II].

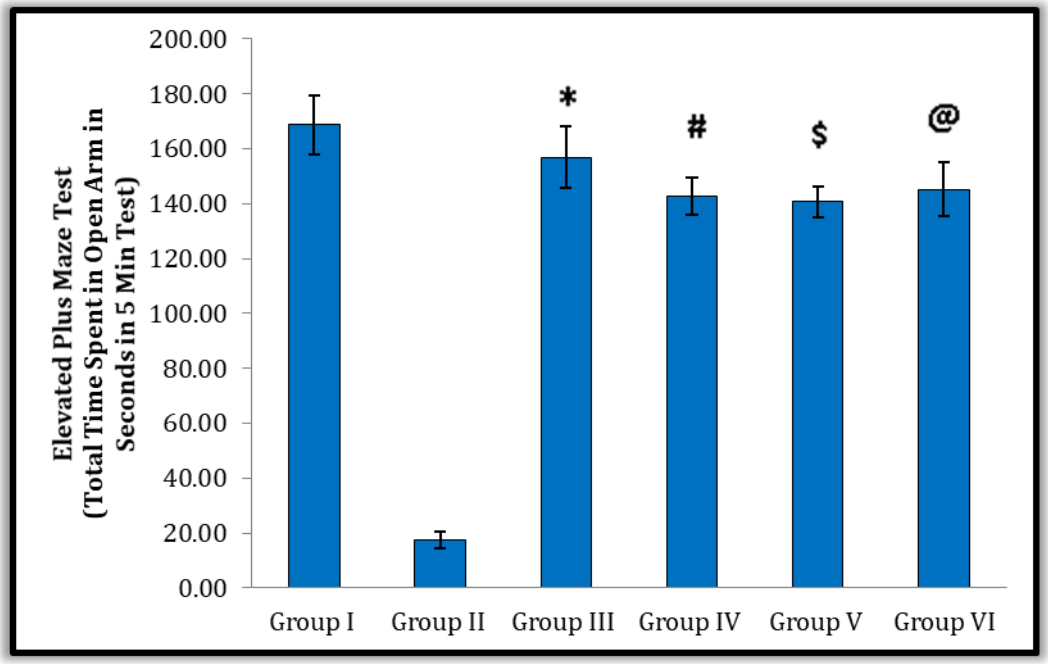


Figure 39: Bar diagram depicting the total time spent in the open arm in elevated plus maze test in Rotenone model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group (Standard drug group) [Group III] to the negative control group [Group II] in the parameter (Time Spent In Open Arm in the Elevated Plus Maze Test), it was noticed that there was a significant (P<0.05) increase in the Levodopa+Benserazide group (Standard drug group) [Group III]. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant (P<0.05) increase in the time spent in the open arm.

Table 12: Behavioural analysis in Rotenone model

Group	Actophotometer test	Rotarod test	Grip strength test	Hole board test	Tail suspension test	Force swim test	Elevated plus maze			
							% Open arm preference	No. of entries into the open arm	No. of entries into the close arm	Time spent in the open arm
I [Vehicle control]	174.33±25.01	118.17±1.83	116.33±3.67	35.50±3.63	26.50±4.30	31.33±3.64	83.33	16.50±1.12	2.67±0.71	168.67±10.73
II [Negative control]	18.67±4.11	10.17±1.19	8.83±1.80	6.33±1.26	179.17±20.26	165.33±9.12	16.67	3.17±0.48	17.83±1.51	17.50±2.86
III [Standard control]*	161.33±15.25	116.17±2.59	113.17±6.83	31.17±3.46	29.67±4.71	34.50±3.00	83.33	14.67±1.58	3.17±0.87	156.83±11.22
IV [Captopril]#	149.83±17.04	110.17±7.01	109.17±6.88	28.50±2.29	37.17±3.11	41.50±1.91	66.67	11.67±1.05	4.17±0.65	142.83±6.73
V [Perindopril]$	141.83±9.16	108.67±6.49	106.83±8.34	25.33±3.28	41.17±3.28	44.33±2.94	50.00	10.50±1.23	4.50±0.76	140.67±5.52
VI [Losartan]@	154.50±15.42	114.17±5.83	111.50±8.50	29.50±3.70	32.33±2.89	38.33±3.39	66.67	12.67±1.20	3.67±0.49	145.17±9.71

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Evaluation of oxidative stress markers

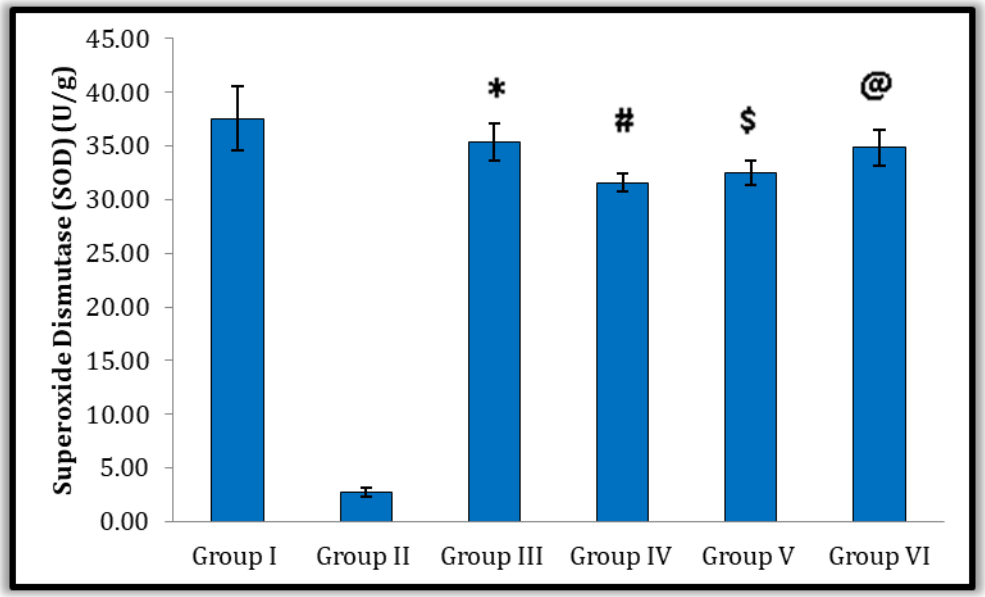


Figure 40: Bar diagram depicting the anti-oxidant enzyme (*Superoxide dismutase*) level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Superoxide dismutase) levels in the negative control group [Group II] were lower (P<0.05). The results revealed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had increased the anti-oxidant enzyme (*Superoxide dismutase*) levels and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

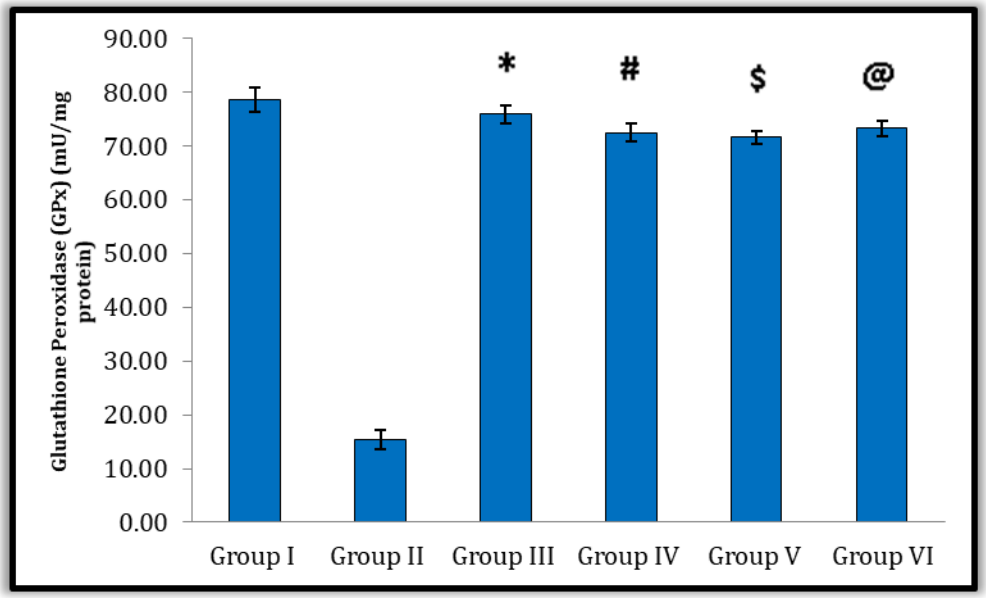


Figure 41: Bar diagram depicting the anti-oxidant enzyme (*Glutathione peroxidase*) level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the anti-oxidant enzyme (Glutathione peroxidase) level in negative

control group was reduced (P<0.05). The study also found that the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increase in the anti-oxidant enzyme (*Glutathione peroxidase*) level and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

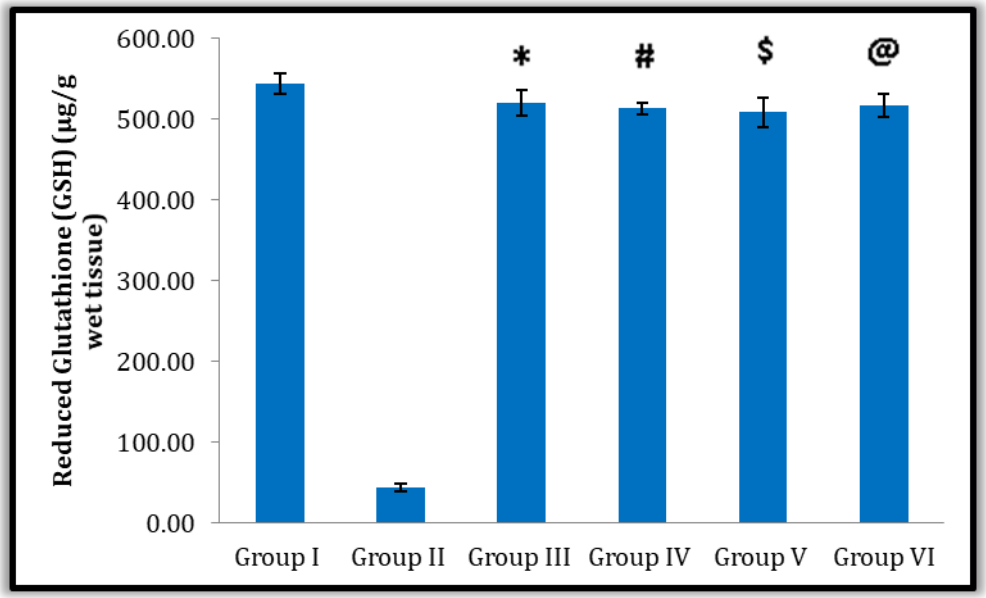


Figure 42: Bar diagram depicting the anti-oxidant enzyme (*Reduced glutathione*) level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (reduced glutathione) level was declined in the negative control group [Group II]. The study also found that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had increased anti-oxidant enzyme (*Reduced glutathione*) level and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

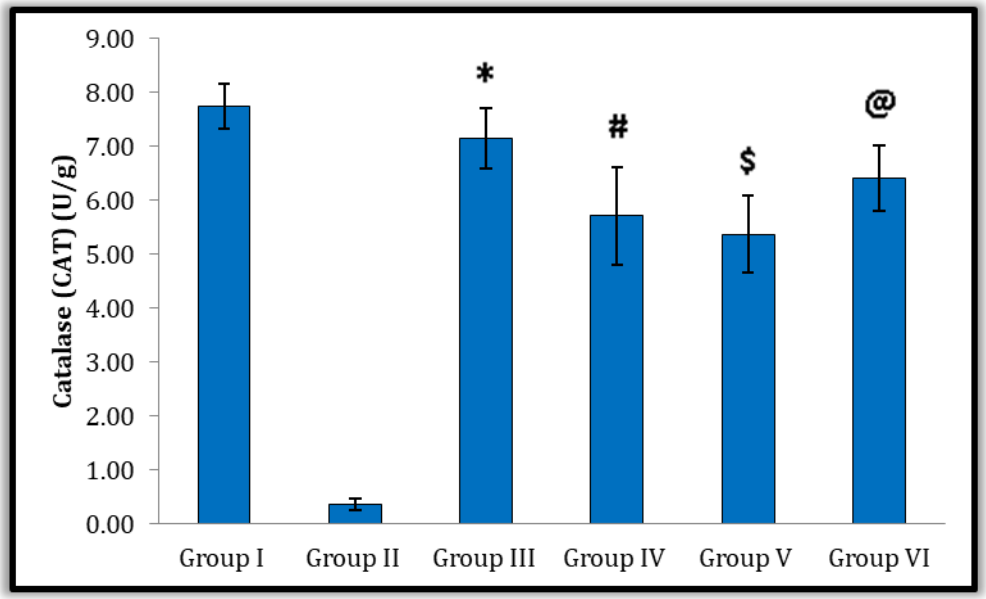


Figure 43: Bar diagram depicting the anti-oxidant enzyme (*Catalase*) level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Catalase) level was reduced significantly (P<0.05) in the negative control group [Group II]. The study also revealed that both the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] had similar outcomes (Captopril, Perindopril and Losartan) depicting an increased levels of anti-oxidant enzyme (*Catalase*) which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

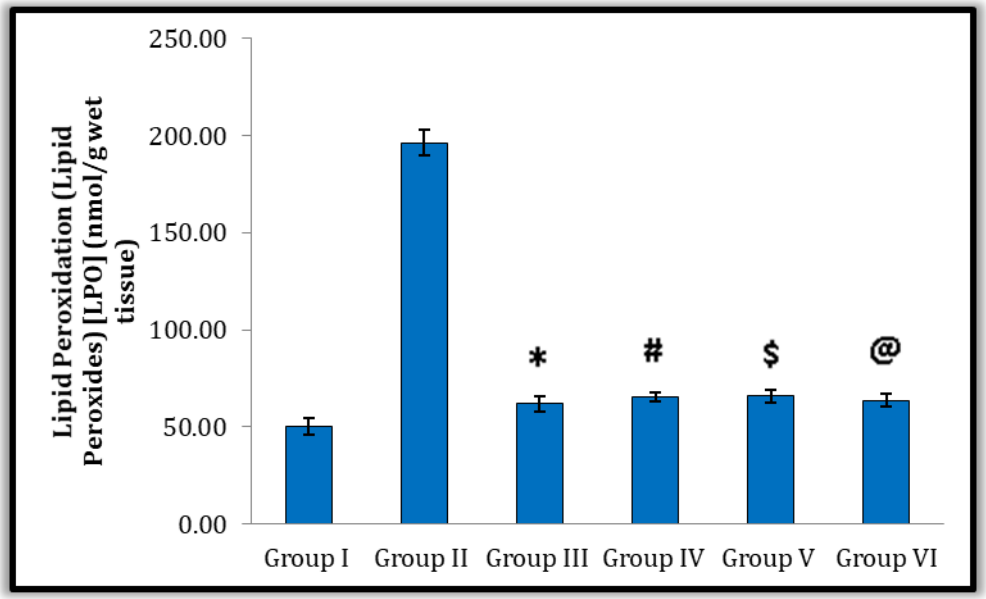


Figure 44: Bar diagram depicting the *lipid peroxidation* level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The lipid peroxidation was significantly increased in the negative control group [Group II] when compared to the vehicle control group [Group I] (P<0.05). The study also found that, standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) indicated a decrease in the lipid peroxidation and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 13: Anti-oxidant enzymes in Rotenone Model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Superoxide Dismutase (SOD) (*U/g*)	37.57±2.98	2.72±0.43	35.34±1.72	31.59±0.83	32.48±1.11	34.83±1.72
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	78.67±2.22	15.38±1.71	75.97±1.63	72.55±1.67	71.63±1.16	73.31±1.47
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	544.48±13.07	43.76±5.13	520.31±15.50	513.69±7.22	508.85±18.61	517.30±14.41
4	Catalase (CAT) (*U/g*)	7.75±0.41	0.36±0.11	7.14±0.56	5.71±0.90	5.37±0.72	6.41±0.60
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	50.23±4.05	196.36±6.57	61.86±4.00	65.51±2.04	66.04±3.25	63.71±3.43

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], all the anti-oxidant enzyme levels (superoxide dismutase, glutathione peroxidase, reduced glutathione and catalase) were significantly (P<0.05) lower in the negative control group [Group II]. The study also discovered that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increase in the anti-oxidant enzyme (*Superoxide Dismutase, Glutathione Peroxidase, Reduced Glutathione and Catalase*) levels and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05). But there was a significant (P<0.05) decrease in the lipid peroxidation in the standard drug group [Group III] and the experimental groups [Group IV, Group V and

Group VI] (Captopril, Perindopril and Losartan) when compared to the negative control group [Group II].

Evaluation of neurotransmitters

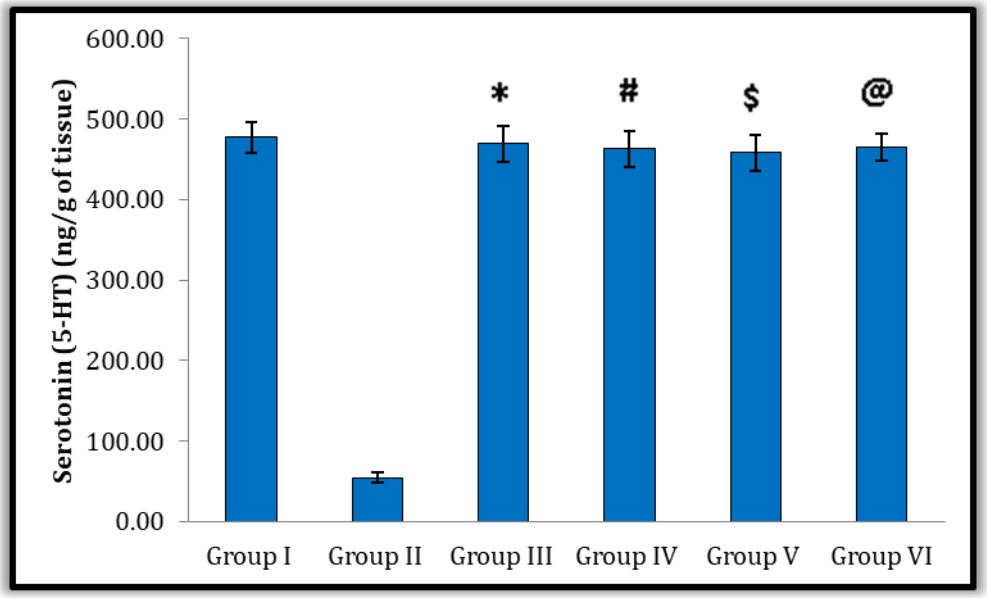


Figure 45: Bar diagram depicting the *Serotonin* level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (serotonin) level in the negative control group [Group II] was lower, and this difference was statistically significant (P<0.05). It was also noticed that the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed an increase in the neurotransmitter (*Serotonin*) level which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

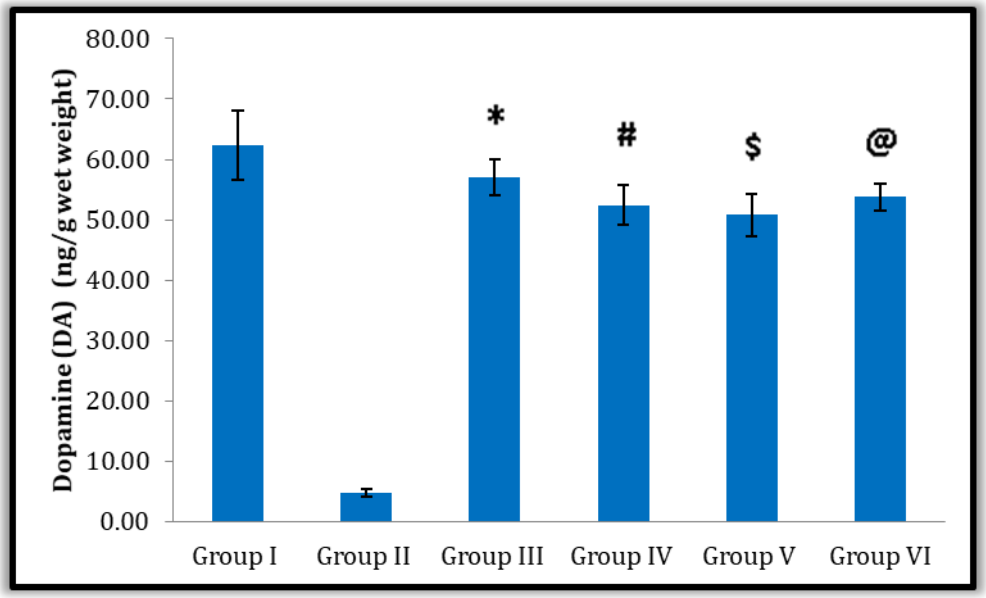


Figure 46: Bar diagram depicting the Dopamine level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (Dopamine) level was reduced in the negative control group [Group II], which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] were also observed (Captopril, Perindopril and Losartan) an increased in the neurotransmitter (*Dopamine*) level which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

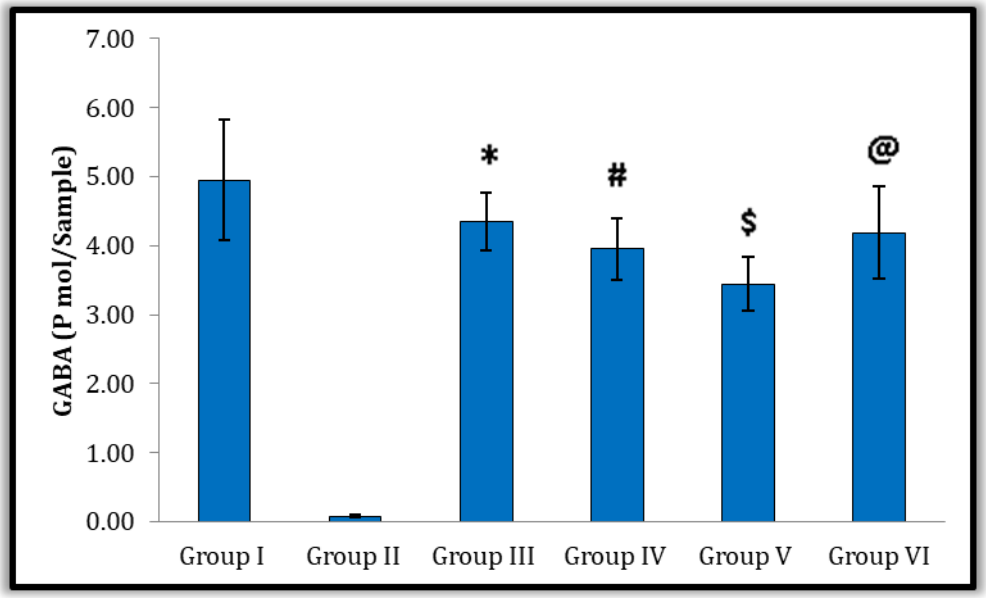


Figure 47: Bar diagram depicting the *GABA* level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (GABA) level was declined in the negative control group [Group II], which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] were also analysed to (Captopril, Perindopril and Losartan) show an increase in the neurotransmitter (*GABA*) level and it was found to be statistically significant when compared to the negative control group [Group III] (P<0.05).

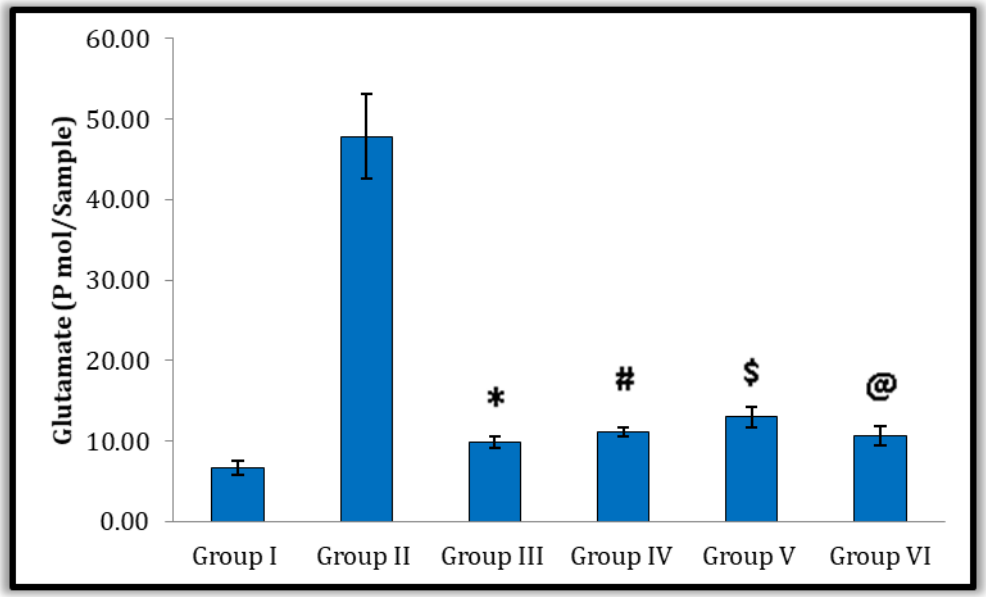


Figure 48: Bar diagram depicting the *Glutamate* level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (*Glutamate*) level was increased in the negative control group [Group II] when compared to the vehicle control group [Group I] and it was found to be statistically significant (P<0.05). It was also seen that, the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) decreased the neurotransmitter (*Glutamate*) level and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

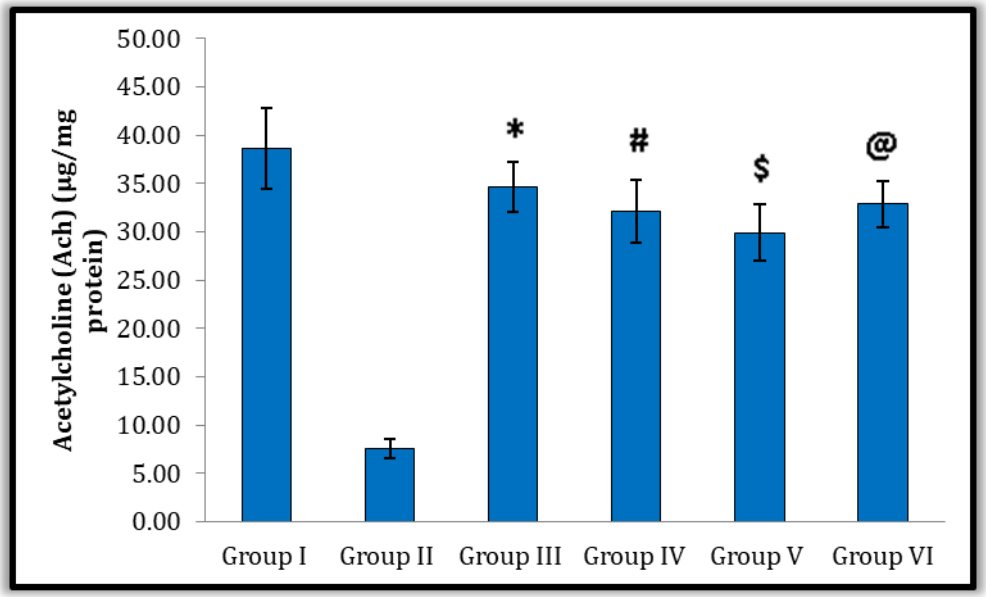


Figure 49: Bar diagram depicting the *Acetylcholine* level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group I] to the vehicle control group [Group I], the neurotransmitter (ACh) level was found to be significantly reduced in the negative control group [Group II], which was statistically significant (P<0.05). It was also revealed that the experimental groups [Group IV, Group V and Group VI (Captopril, Perindopril and Losartan)] as well as the standard drug group [Group III] had an increase in the neurotransmitter (ACh) level which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 14: Neurotransmitters in Rotenone Model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Serotonin (*ng/g of tissue*)	477.28±18.72	54.51±6.16	469.25±21.97	463.12±21.97	458.04±22.75	465.58±16.62
2	Dopamine (*ng/g wet weight*)	62.37±5.70	4.74±0.69	57.05±2.90	52.43±3.27	50.82±3.51	53.83±2.23
3	GABA (*P mol/Sample*)	4.95±0.88	0.08±0.02	4.35±0.42	3.95±0.45	3.44±0.39	4.19±0.67
4	Glutamate (*P mol/Sample*)	6.68±0.81	47.86±5.23	9.87±0.71	11.10±0.59	13.04±1.27	10.65±1.22
5	Acetylcholine (*µg/mg protein*)	38.64±4.24	7.58±1.02	34.63±2.61	32.13±3.20	29.92±2.93	32.86±2.34

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (5-HT, DA, GABA, and Ach) levels were decreased in the negative control group [Group II], which was statistically significant (P<0.05). It was also noticed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had shown an increase in the neurotransmitter (*5-HT, DA and GABA*) levels and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

When comparing the negative control group [Group II] to the vehicle control group [Group I], the neurotransmitter (glutamate) level was increased in negative control group and shown to be statistically significant (P<0.05. The standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI (Captopril, Perindopril and Losartan) were also observed a decreased neurotransmitter (*Glutamate*) level which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Evaluation of inflammatory marker

Myeloperoxidase [MPO]

Table 15: Evaluation of inflammatory marker [MPO] in Rotenone model

Group	I [Vehicle control]	II [Negative control]	III [Standard control]	IV [Captopril]	V [Perindopril]	VI [Losartan]
MPO	0.44± 0.05	13.12 ±1.33	0.95±0. 26	1.79±0.29	2.29±0.59	1.28±0. 25

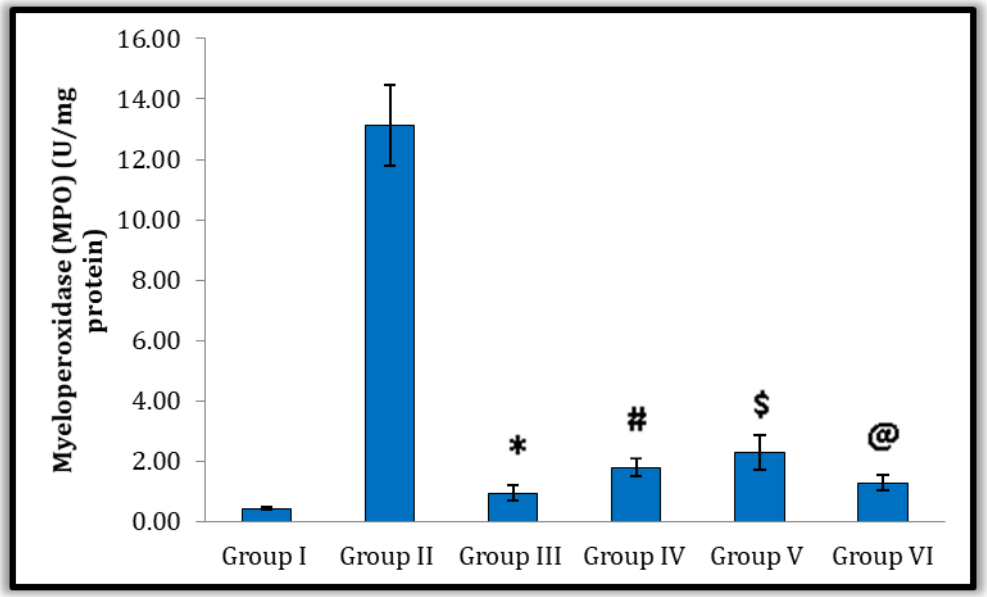


Figure 50: Bar diagram depicting the inflammatory marker (*Myeloperoxidase*) level in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had a higher level of myeloperoxidase, which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a decreased Myeloperoxidase level which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

MICROANATOMICAL [HISTOPATHOLOGICAL] STUDY IN ROTENONE MODEL

Microanatomical [Histopathological] study of Hippocampus in Rotenone model

Histology of normal Hippocampus

Three layered architecture was appreciated. The layers are:

- Layer 1 – Molecular layer with nerve fibres and small cell bodies
- Layer 2 – Granular cell layer
- Layer 3 – Polymorphic layer with pyramidal cell dendrites

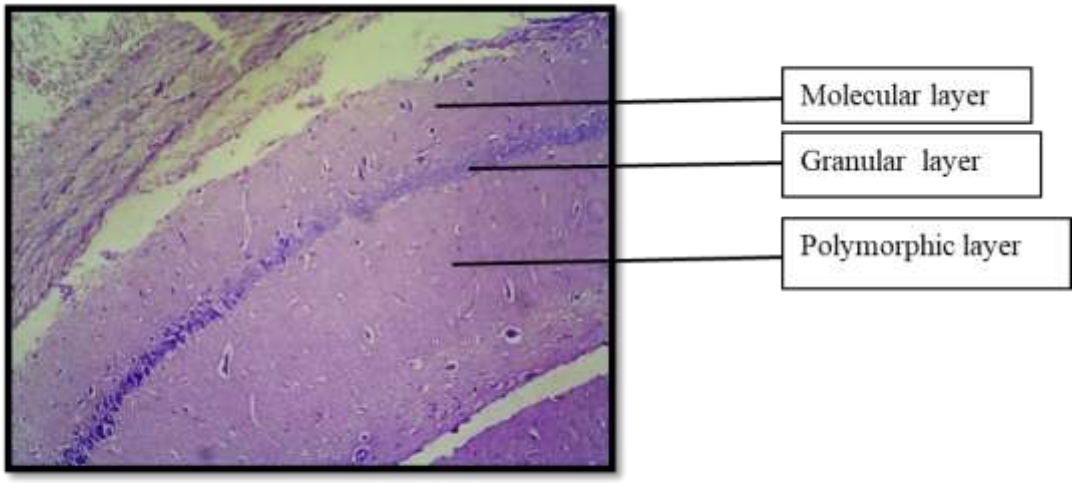
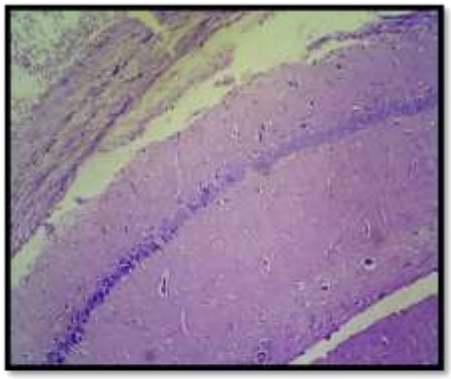


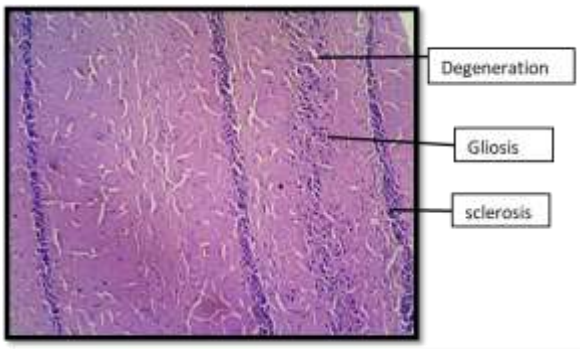
Figure 51: Section of rat brain showing normal Hippocampus (10x; H & E stained)



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 52 : Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Rotenone model** (10x; H & E stained)

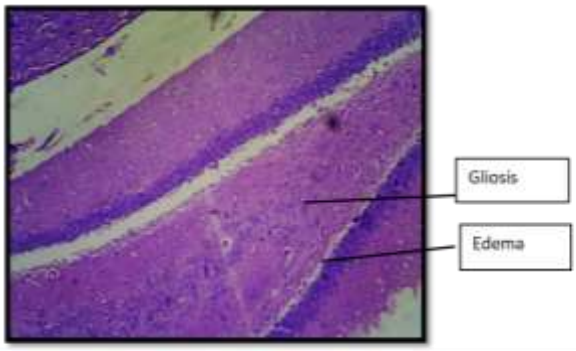


Section studied from the rat brain [Hippocampus] of Rotenone alone group [Group II] showing severe gliosis, sclerosis and degeneration amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 53: Section of rat brain [**Hippocampus**] **Rotenone alone group [Group II]** in **Rotenone model**

(10x; H & E stained)

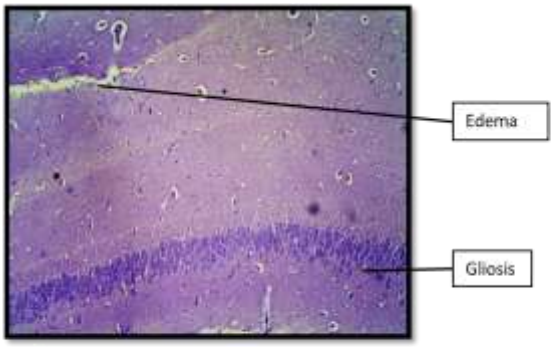


Section studied from the rat brain [Hippocampus] of Rotenone + Positive control group [Group III] showing mild gliosis and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 54: Section of rat brain [**Hippocampus**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model**

(10x; H & E stained)



Section studied from the rat brain [Hippocampus] of Rotenone + Captopril group [Group IV] showing gliosis & edema amounting to the histopathological score 1.50*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 55 : Section of rat brain [**Hippocampus**] from **Rotenone + Captopril [Group IV]** in **Rotenone model**

(10x; H & E stained)

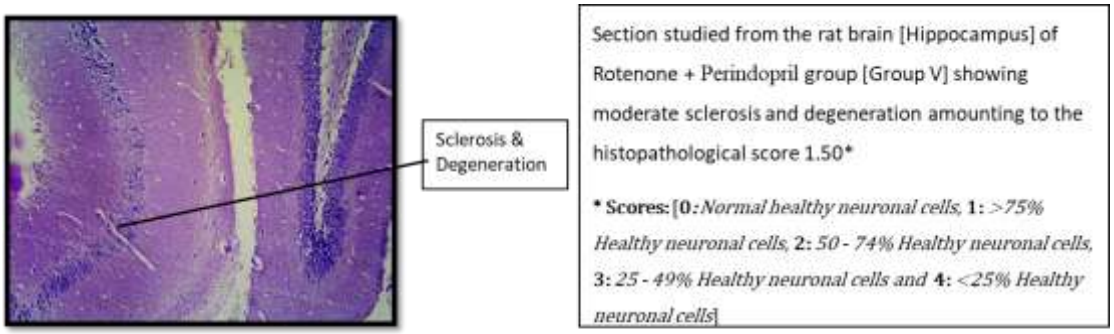


Figure 56 : Section of rat brain [**Hippocampus**] from **Rotenone + Perindopril [Group V]** in **Rotenone model**
(10x; H & E stained)

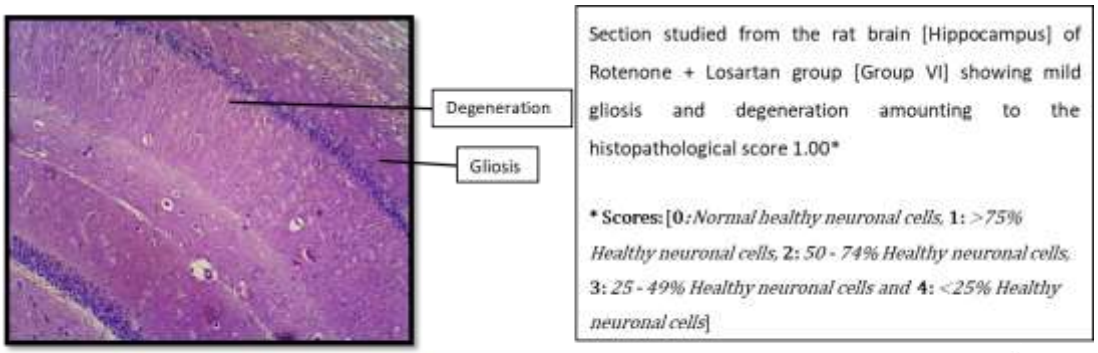


Figure 57: Section of rat brain [**Hippocampus**] from **Rotenone + Losartan [Group VI]** in **Rotenone model**
(10x; H & E stained)

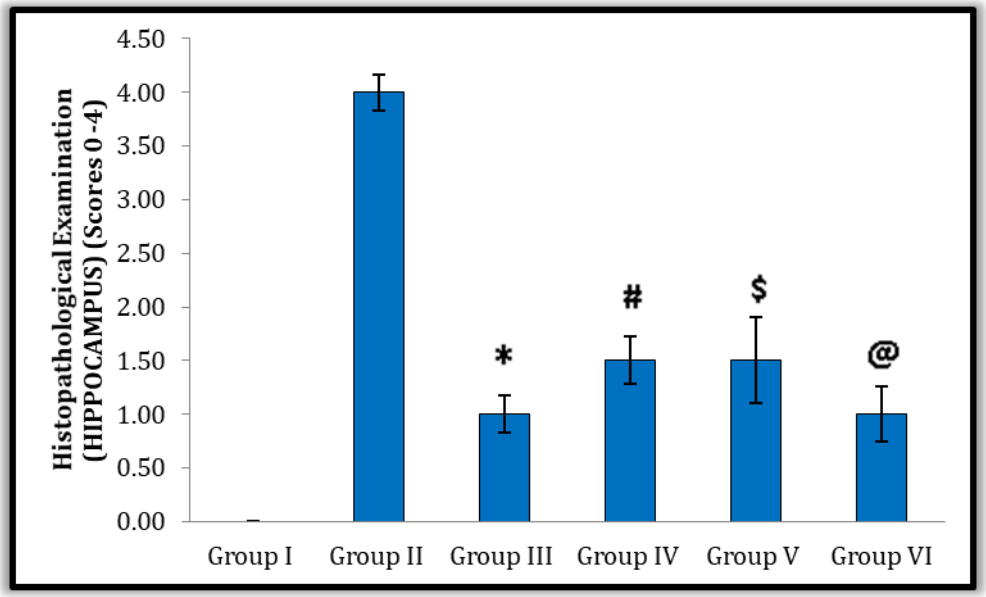


Figure 58: Bar diagram depicting the histopathological examination scores in Hippocampus in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hippocampus, which was statistically significant (P<0.05). The standard drug group [Group III] as well as the experimental groups [Group IV, Group V, and Group VI] (Captopril, Perindopril, and Losartan) found to show decreased histopathological scores in the hippocampus which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Microanatomical [Histopathological] examination of Prefrontal cortex [Cerebrum] in Rotenone model

Cerebrum: Showed normal six layers. The layers are:

- Layer I – Molecular layer
- Layer II – External granular layer
- Layer III – External pyramidal cell layer
- Layer IV – Internal granular layer
- Layer V – Internal pyramidal layer
- Layer VI – Multiform layer

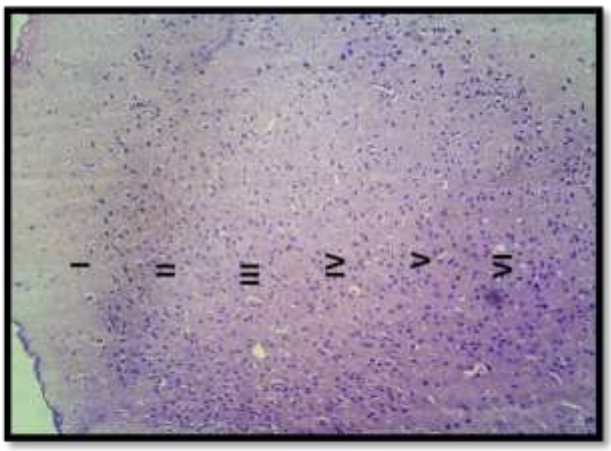
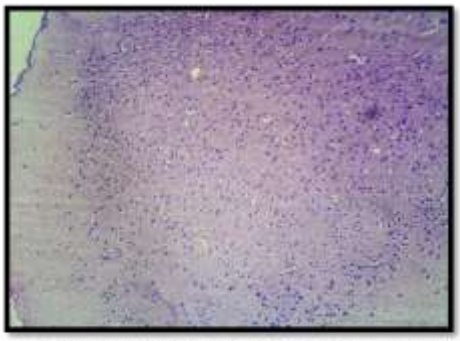


Figure 59: Section of rat brain showing normal Prefrontal cortex (10x; H & E stained)

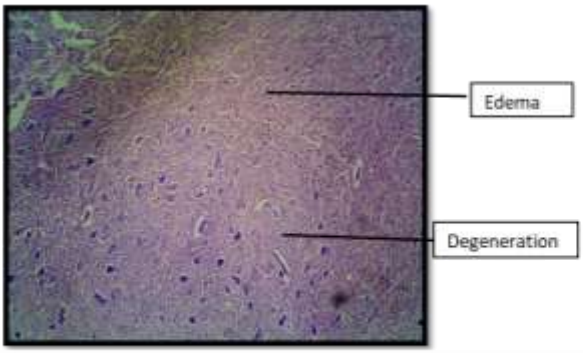


Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 60: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Rotenone model**

(10x; H and E stained)

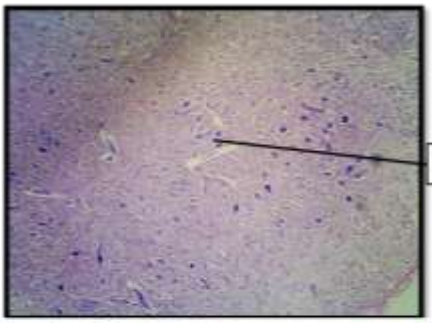


Section studied from the rat brain [Prefrontal cortex] of Rotenone alone group [Group II] showing severe edema and degeneration amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 61: Section of rat brain [**Prefrontal cortex**] from **Rotenone alone group [Group II]** in **Rotenone model**

(40x; H and E stained)

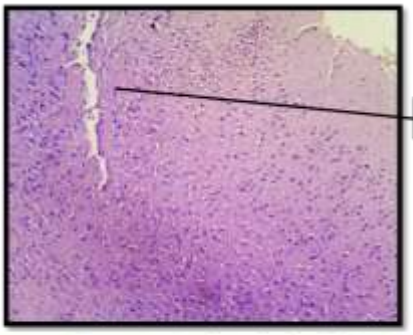


Section studied from the rat brain [Prefrontal cortex] of Rotenone + Positive control group [Group III] showing mild degeneration and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 62: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model**

(10x; H and E stained)

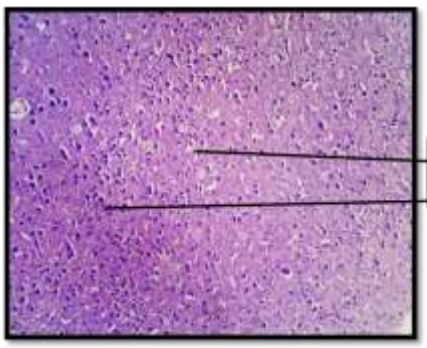


Section studied from the rat brain [Prefrontal cortex] of Rotenone + Captopril [Group IV] showing moderate degeneration amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 63: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Captopril [Group IV]** in **Rotenone model**

(10x; H and E stained)

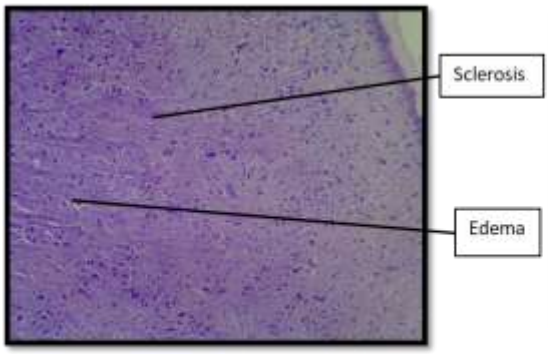


Section studied from the rat brain [Prefrontal cortex] of Rotenone + Perindopril group [Group V] showing Hippocampal degeneration and edema amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 64 : Section of rat brain [**Prefrontal cortex**] from **Rotenone + Perindopril [Group V]** in **Rotenone model**

(10x; H and E stained)



Section studied from the rat brain [Corpus striatum] of Rotenone + Losartan group [Group VI] showing sclerosis and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 65 : Section of rat brain [**Prefrontal cortex**] from **Rotenone + Losartan** [Group VI] in **Rotenone model** (10x; H and E stained)

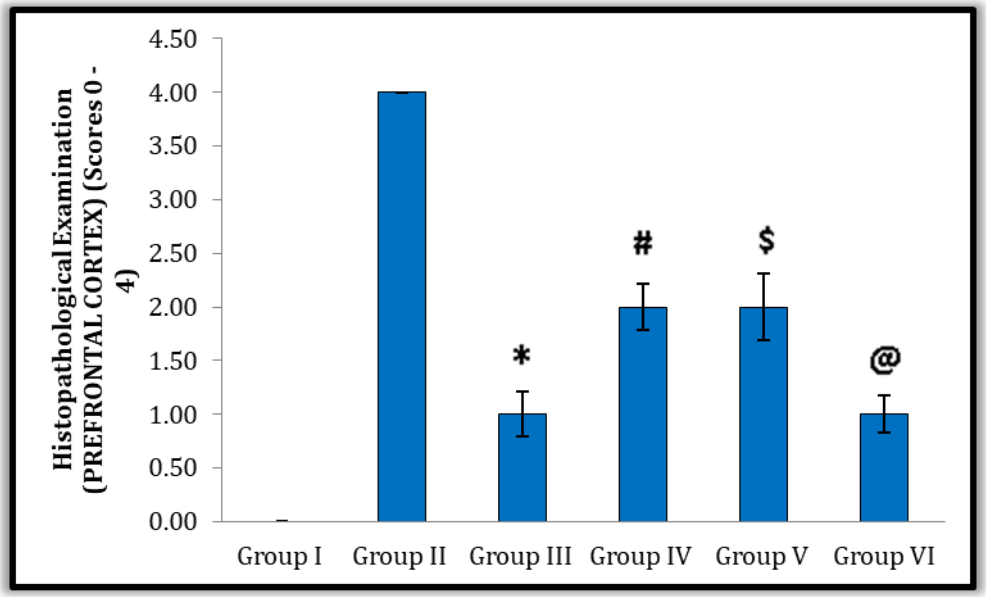


Figure 66: Bar diagram depicting the histopathological examination scores in Prefrontal cortex in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the prefrontal cortex, which was statistically significant (P<0.05). The standard drug group [Group III] as well as the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a decrease in the histopathological scores in the prefrontal

cortex which was found to be statistically significant when compared to the negative control group [Group II] ($P<0.05$).

Microanatomical [Histopathological] examination of Corpus striatum [Basal nuclei] in Rotenone model

Corpus striatum: Heterogeneous mixture of neuronal cell bodies and fibres appreciated

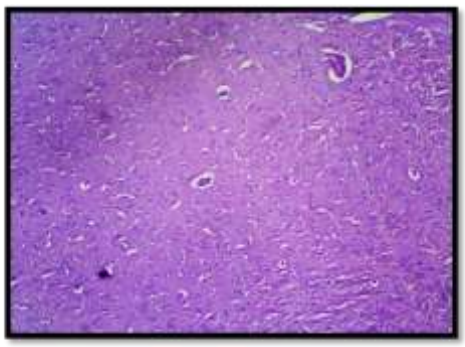
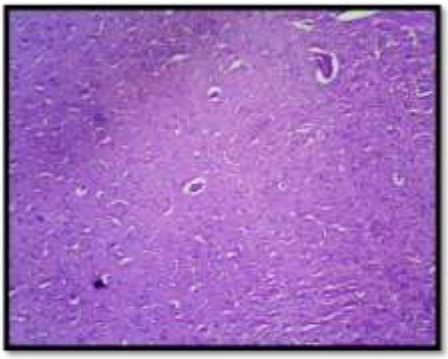


Figure 67 : Section of rat brain showing normal Corpus striatum
(10x; H and E stained)



Section studied from the mouse brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 68: Section of mouse brain [Corpus striatum] from **Vehicle control group [Group I]** in Rotenone Model
(10x; H & E stained)

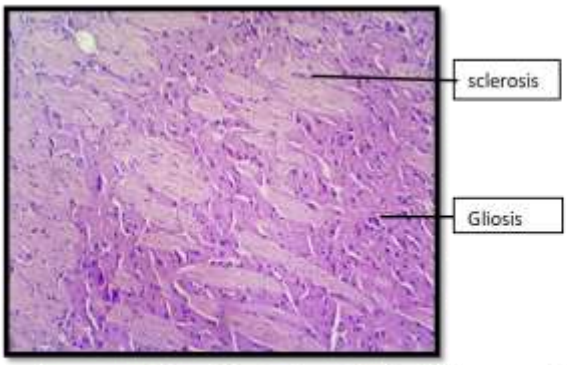


Figure 69 : Section of rat brain **[Corpus striatum]** from **Rotenone alone group [Group II]** (40x; H & E stained)

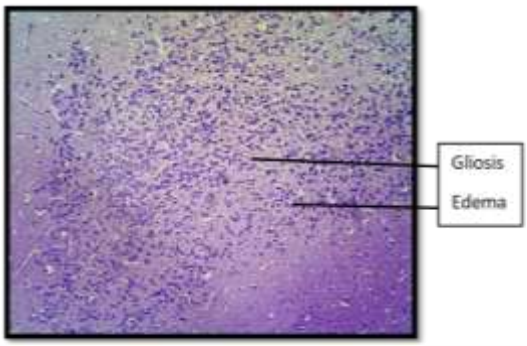


Figure 70: Section of rat brain **[Corpus striatum]** from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model** (10x; H & E stained)

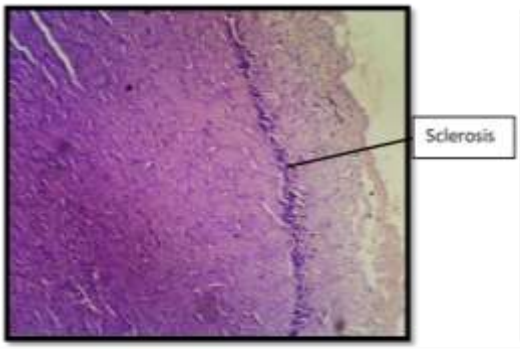


Figure 71 : Section of rat brain **[Corpus striatum]** from **Rotenone + Captopril [Group IV]** in **Rotenone model** (10x; H & E stained)

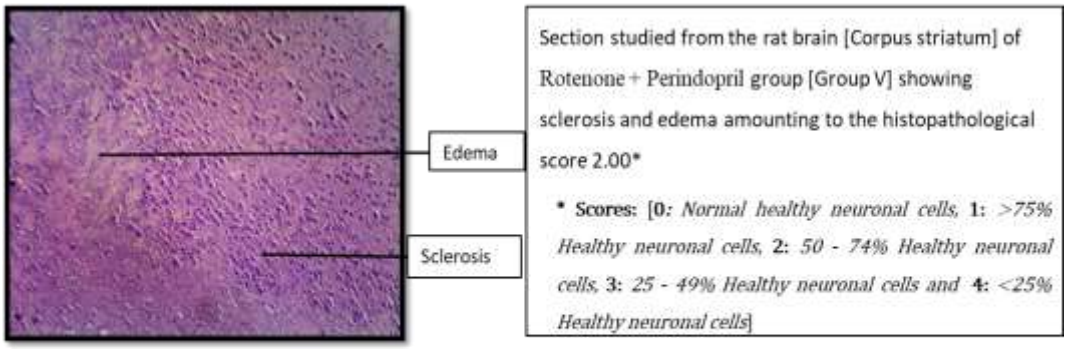


Figure 72: Section of rat brain **[Corpus striatum]** from **Rotenone + Perindopril** [Group V] in **Rotenone model**

(10x; H and E stained)

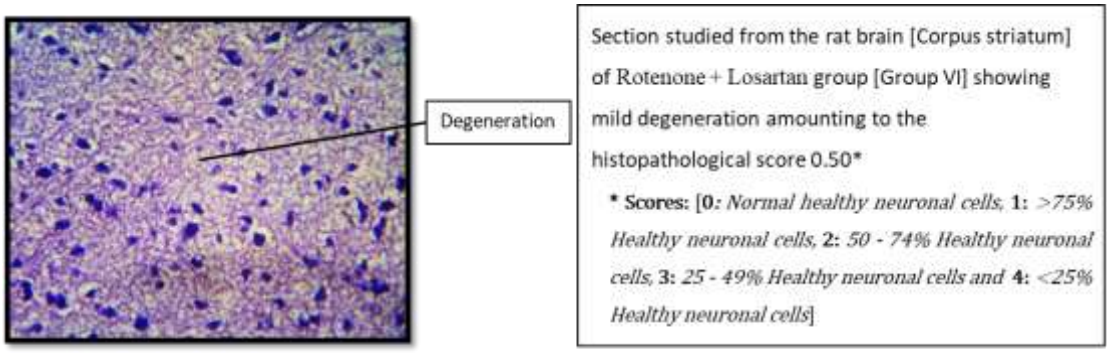


Figure 73: Section of rat brain **[Corpus striatum]** from **Rotenone + Losartan** [Group VI] in **Rotenone model**

(40x; H &E stained)

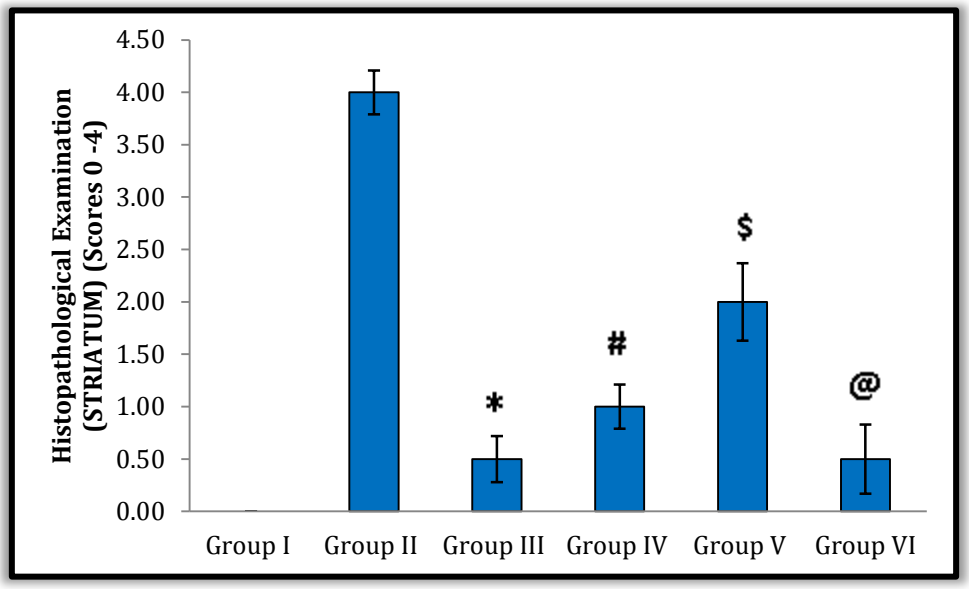


Figure 74: Bar diagram depicting the histopathological examination scores in the corpus striatum in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the histopathological scores in the corpus striatum were significantly higher in the negative control group [Group II], which was statistically significant (P<0.05). It was also observed that the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as well as the standard drug group [Group III] had a decreased histopathological scores in the corpus striatum which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Microanatomical [Histopathological] examination of Hypothalamus in Rotenone model

Hypothalamus: Heterogeneous mixture of neuronal cell bodies and fibres appreciated

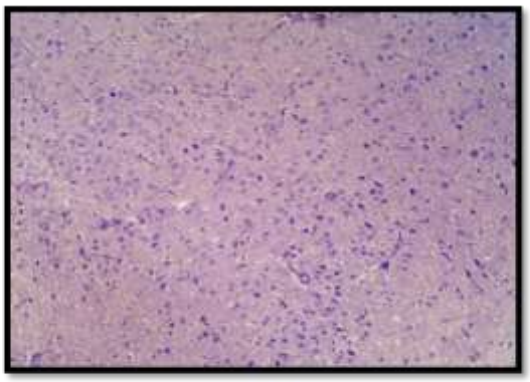
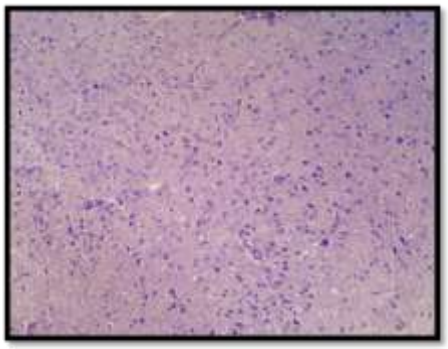


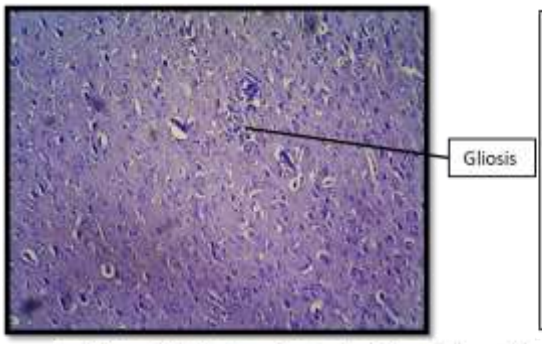
Figure 75: Section of rat brain showing normal Hypothalamus (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

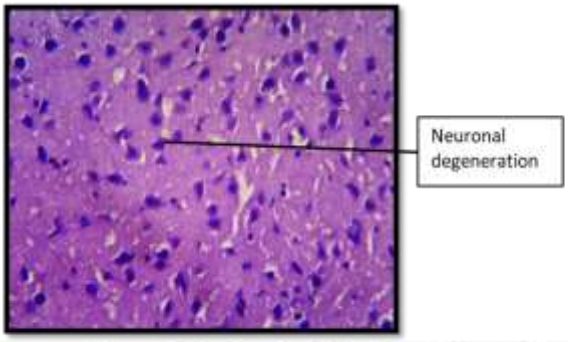
Figure 76: Section of rat brain [Hypothalamus] from **Vehicle control group [Group I]** in **Rotenone model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Rotenone alone group [Group II] showing gliosis amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

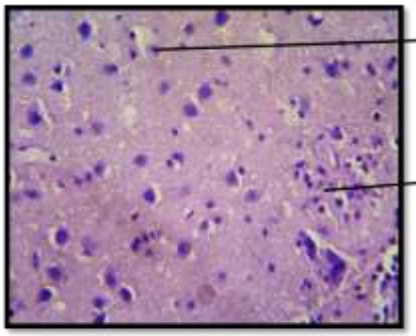
Figure 77: Section of rat brain [Hypothalamus] from Rotenone **alone group [Group II]** in **Rotenone model** (10x; H & E stained)



Section studied from the rat brain [Hypothalamus] of Rotenone + Positive control group [Group III] showing mild neuronal degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 78: Section of rat brain [**Hypothalamus**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model** (40x; H & E stained)

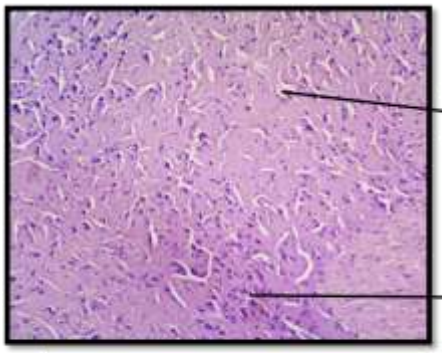


Section studied from the rat brain [Hypothalamus] of Rotenone + Captopril group [Group IV] showing vacoulations and inflammatory infiltrates amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 79: Section of rat brain [**Hypothalamus**] from **Rotenone + Captopril [Group IV]** in **Rotenone model**

(40x; H and E stained)

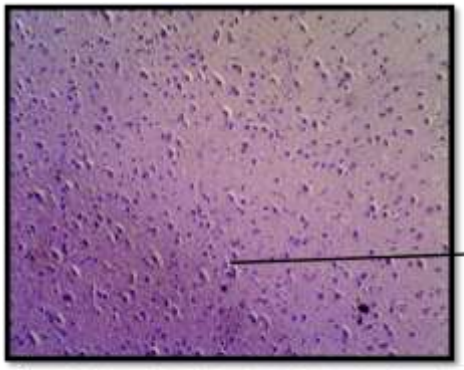


Section studied from the rat brain [Hypothalamus] of Rotenone Perindopril group [Group V] showing gliosis and edema amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 80: Section of rat brain [**Hypothalamus**] from **Rotenone + Perindopril** [Group V] in **Rotenone model**

(10x; H & E stained)



Section studied from the rat brain [Hypotalamus] of Rotenone + Losartan group [Group VI] showing sclerosis and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 81 : Section of rat brain [**Hypothalamus**] from **Rotenone + Losartan** [Group VI] in **Rotenone model**

(10x; H & E stained)

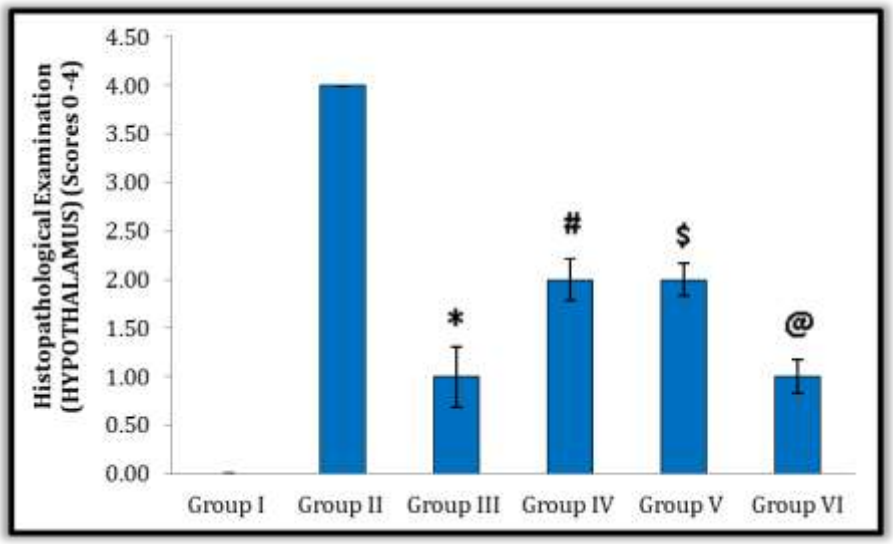


Figure 82: Bar diagram depicting the histopathological examination scores in the Hypothalamus in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hypothalamus, which was statistically significant (P<0.05). The standard drug group [Group III] as well as the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a decreased histopathological scores in the hypothalamus and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 16: Histopathological examination scores in Rotenone Model screening test in wistar albino rats

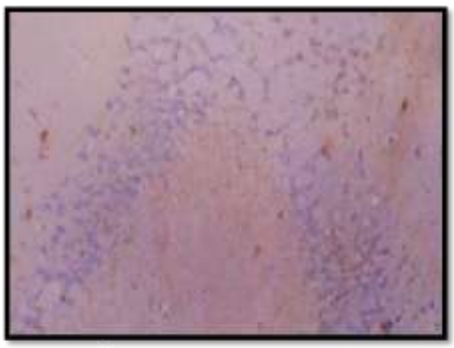
Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0-4)	0.00±0.00	4.00±0.17	1.00±0.17	1.50±0.22	1.50±0.40	1.00±0.26
2	Prefrontal Cortex (Scores 0-4)	0.00±0.00	4.00±0.00	1.00±0.21	2.00±0.21	2.00±0.31	1.00±0.17
3	Corpus Striatum (Scores 0-4)	0.00±0.00	4.00±0.21	0.50±0.22	1.00±0.21	2.00±0.37	0.50±0.33
4	Hypothalamus (Scores 0-4)	0.00±0.00	4.00±0.00	1.00±0.31	2.00±0.21	2.00±0.17	1.00±0.17

Data are represented as Median ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus. This difference was statistically significant (P<0.05). It was also found that the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as well as the standard drug group [Group III] showed a decreased histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and the hypothalamus which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

EVALUATION OF IMMUNOHISTOCHEMISTRY IN ROTENONE MODEL

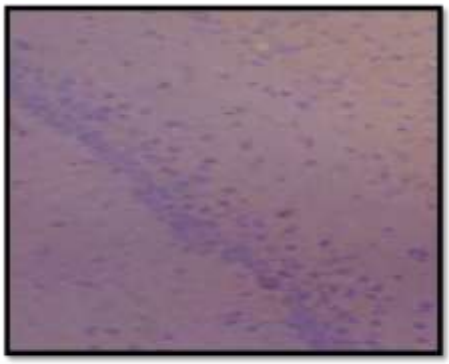
Immunohistochemistry of Hippocampus in various groups of Rotenone model



Section studied from the rat brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

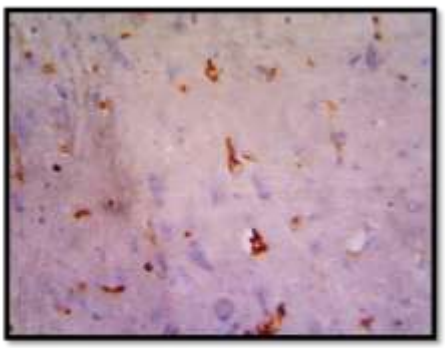
Figure 83: Section of rat brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Hippocampus] of Rotenone alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

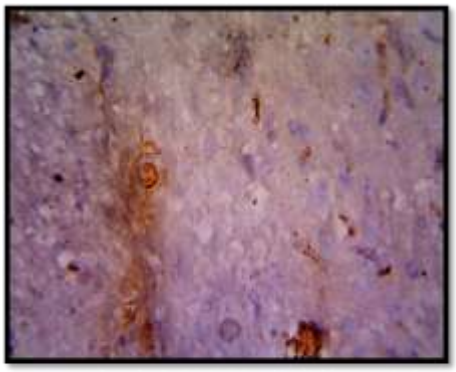
Figure 84 : Section of rat brain [**Hippocampus**] from **Rotenone alone group [Group II]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Hippocampus] of Rotenone + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

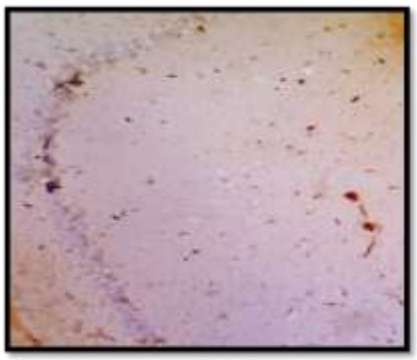
Figure 85 : Section of rat brain [**Hippocampus**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [hippocampus] of Rotenone + Captopril [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

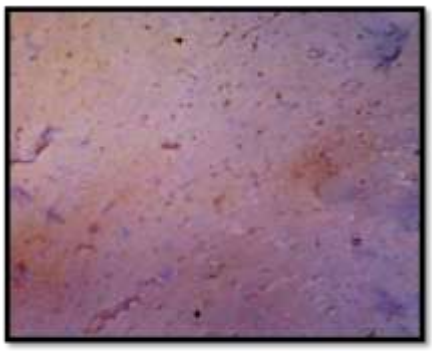
Figure 86 : Section of rat brain [**Hippocampus**] from **Rotenone + Captopril [Group IV]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Hippocampus] of PTZ + Nimodipine group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 87 : Section of rat brain [**Hippocampus**] from **Rotenone + Perindopril [Group V]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Hippocampus] of Rotenone + Losartan [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.0 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 88 : Section of rat brain [**Hippocampus**] from **Rotenone + Losartan [Group VI]** in **Rotenone model** (10x; IHC Bcl-2)

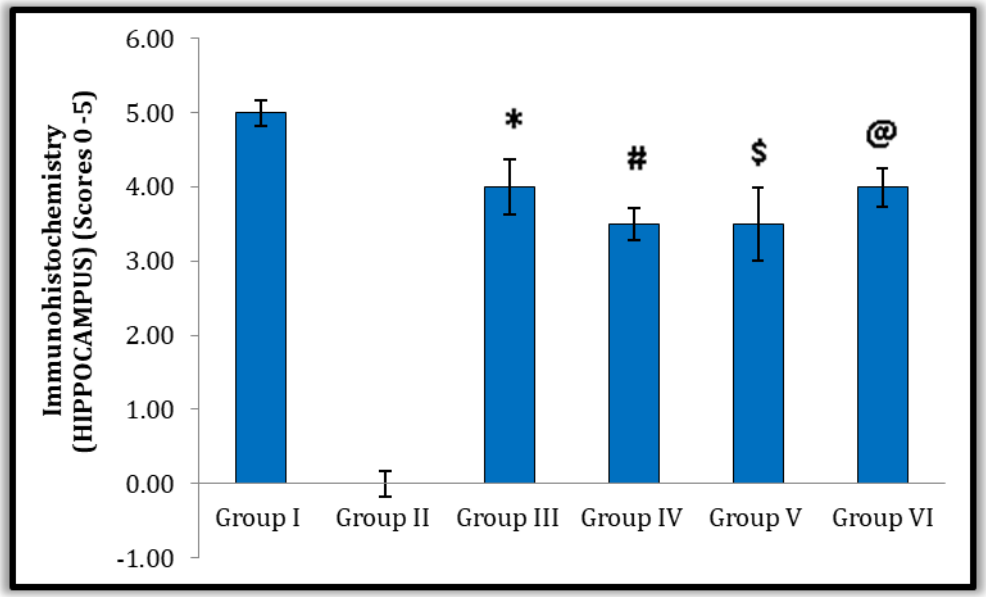
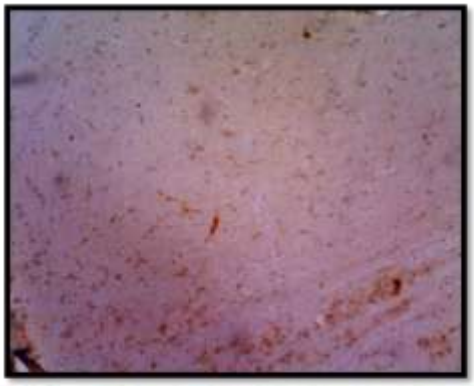


Figure 89: Bar diagram depicting the immunohistochemistry examination scores in the Hippocampus in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores in the hippocampus were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Additionally, the standard drug group [[Group III] and the experimental drug groups [Group IV, Group V and Group VI] Captopril, Perindopril and Losartan) had an increase in the IHC scores in the hippocampus which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

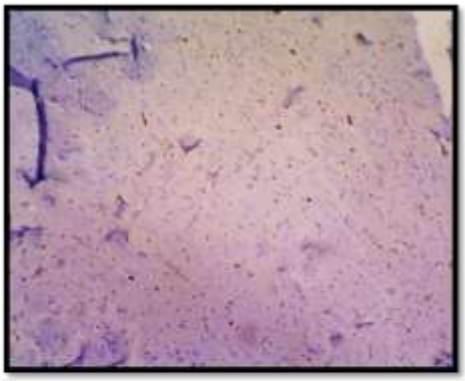
Immunohistochemistry of Prefronal cortex [Cerebrum] in various groups of Rotenone model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

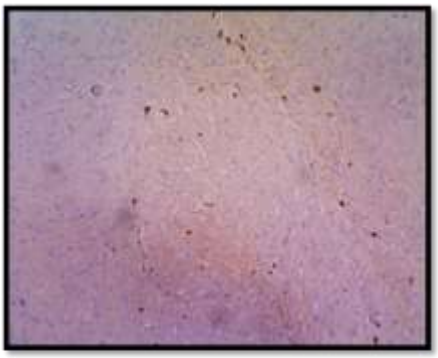
Figure 90: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Prefrontal cortex] of Rotenone alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

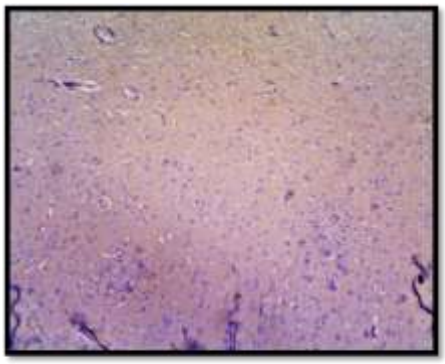
Figure 91: Section of rat brain [**Prefrontal cortex**] from **Rotenone alone group [Group II]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Prefrontal cortex] of Rotenone + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

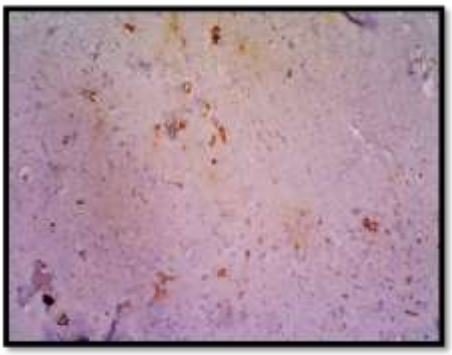
Figure 92: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Prefrontal cortex] of Rotenone + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

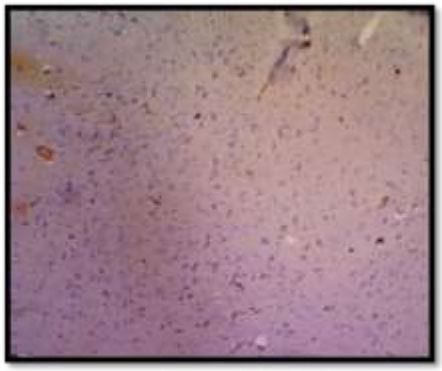
Figure 93: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Captopril [Group IV]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Prefrontal cortex] of Rotenone + Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 94: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Perindopril [Group V]** in **Rotenone model** (10x; IHC Bcl-2)



Section studied from the rat brain [Prefrontal cortex] of Rotenone + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 95: Section of rat brain [**Prefrontal cortex**] from **Rotenone + Losartan [Group VI]** in **Rotenone model** (10x; IHC Bcl-2)

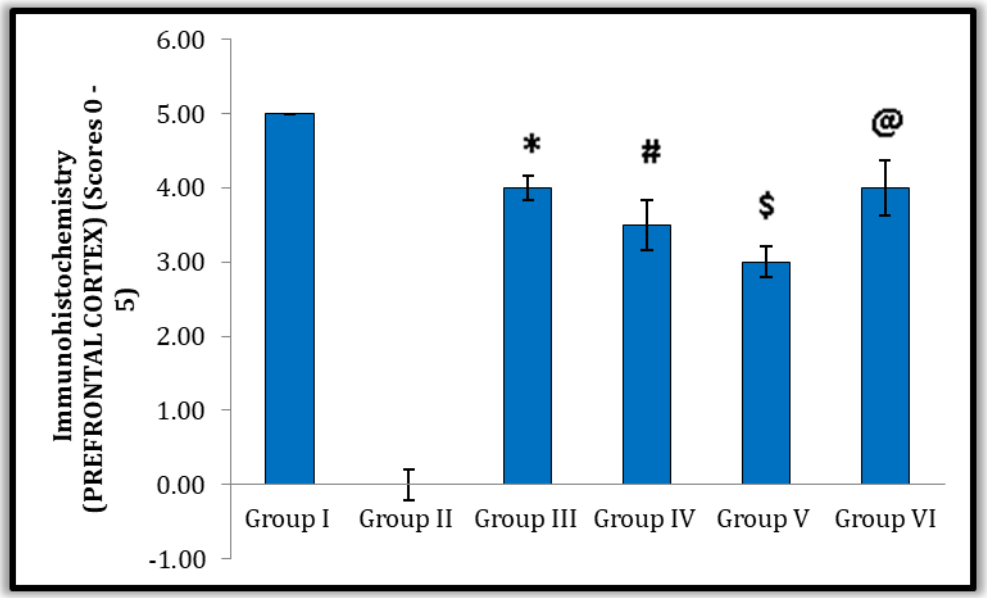
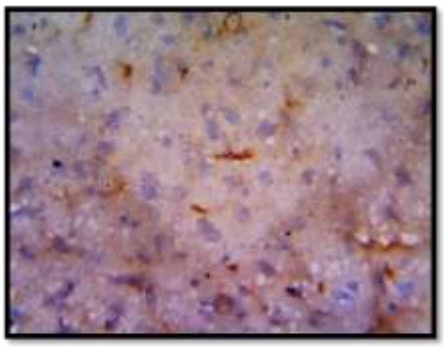


Figure 96: Bar diagram depicting the immunohistochemistry examination scores in Prefrontal cortex in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the prefrontal cortex were lower in the negative control group [Group II], which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had shown an increase in the IHC scores in the prefrontal cortex which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

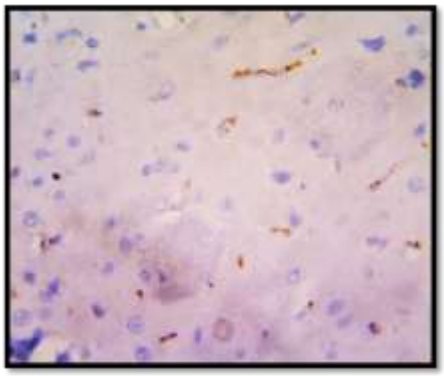
Immunohistochemistry of Corpus striatum [Basal nuclei] in various groups of Rotenone model



Section studied from the rat brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

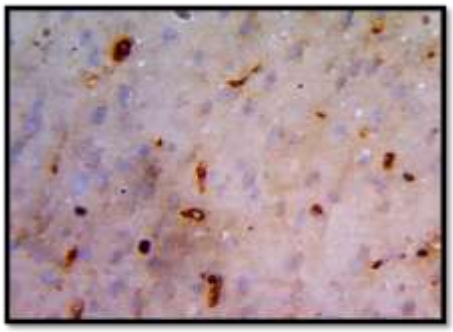
Figure 97: Section of rat brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Corpus striatum] of Rotenone alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

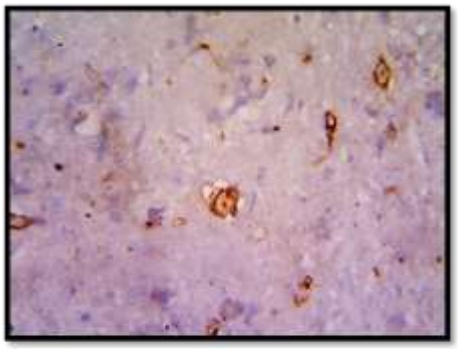
Figure 98: Section of rat brain [**Corpus striatum**] from **Rotenone alone group [Group II]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Corpus striatum] of Rotenone + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

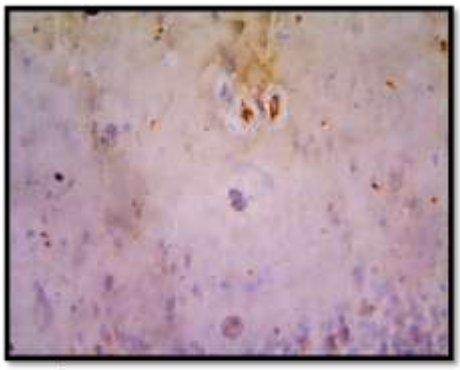
Figure 99: Section of rat brain [**Corpus striatum**] from **Rotenone + Levodopa & Benserazide [Group III]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Corpus striatum] of PTZ + Diltiazem group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

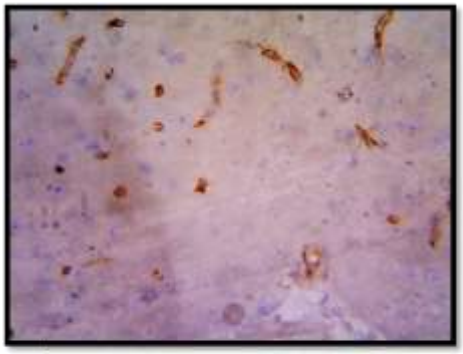
Figure 100: Section of rat brain [**Corpus striatum**] from **Rotenone + Captopril [Group IV]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Corpus striatum] of Rotenone + Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

Figure 101: Section of rat brain [**Corpus striatum**] from **Rotenone + Perindopril [Group V]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Corpus striatum] of Rotenone + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

Figure 102: Section of rat brain [**Corpus striatum**] from **Rotenone + Losartan [Group VI]** in **Rotenone model** (40x; IHC Bcl-2)

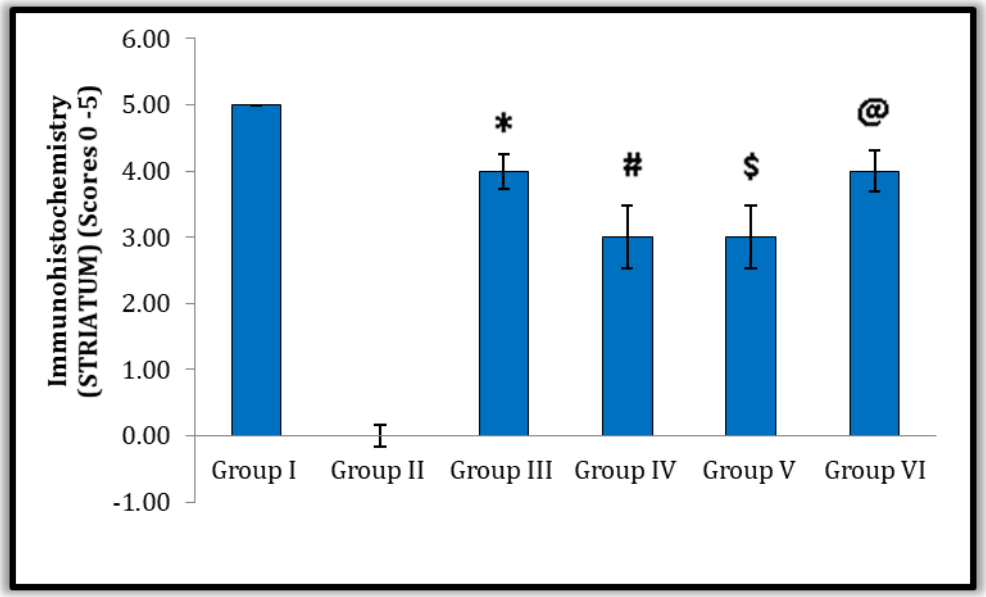
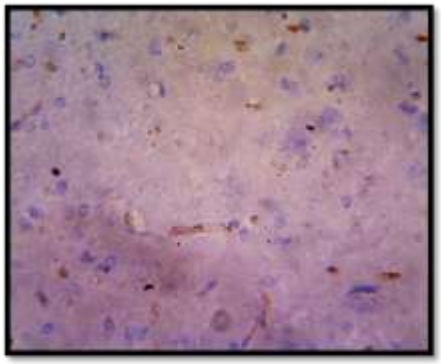


Figure 103: Bar diagram depicting the immunohistochemistry examination scores in Corpus striatum in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the immunohistochemistry scores in the corpus striatum were lower in the negative control group [Group II], which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had an increased IHC scores in the corpus striatum which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

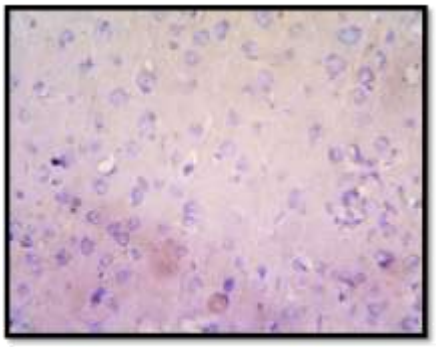
Immunohistochemistry of Hypothalamus in various groups of Rotenone model



Section studied from the rat brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

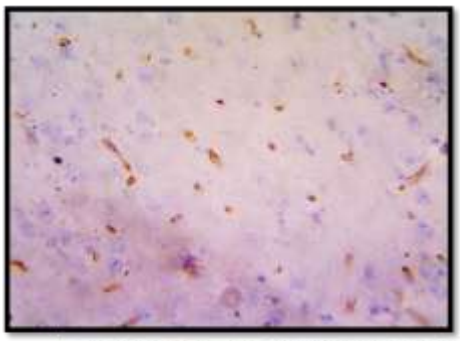
Figure 104 : Section of rat brain [**Hypothalamus**] from **Vehicle control group [Group I] in Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Hypothalamus] of Rotenone alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

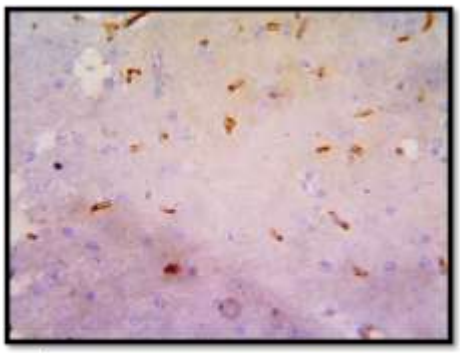
Figure 105: Section of rat brain [**Hypothalamus**] from **Rotenone alone group [Group II]** in **Rotenone model** (40x; IHC Bcl-2)



Section studied from the rat brain [Hypothalamus] of Rotenone + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 106: Section of rat brain [**Hypothalamus**] from **Rotenone + Levadopa & Bensarazide [Group III] in Rotenone model** (40x; IHC Bcl-2)

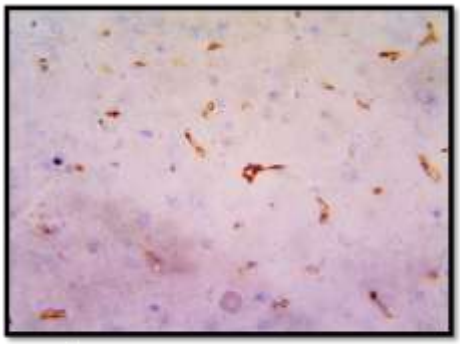


Section studied from the rat brain [Hypothalamus] of **Rotenone + Captopril** group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 107: Section of rat brain [**Hypothalamus**] from **Rotenone + Captopril [Group IV] in Rotenone model**

(40x; IHC Bcl-2)

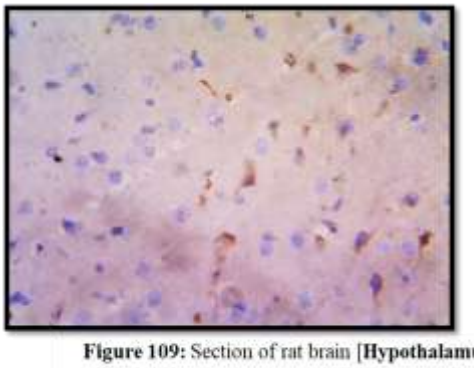


Section studied from the rat brain [Hypothalamus] of Rotenone + Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 2.50 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 108: Section of rat brain [**Hypothalamus**] from **Rotenone + Perindopril [Group V] in Rotenone model**

(40x; IHC HSP70)



Section studied from the rat brain [Hypothalamus] of Rotenone + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 109: Section of rat brain [**Hypothalamus**] from **Rotenone + Losartan [Group VI] in Rotenone model**

(40x; IHC Bcl-2)

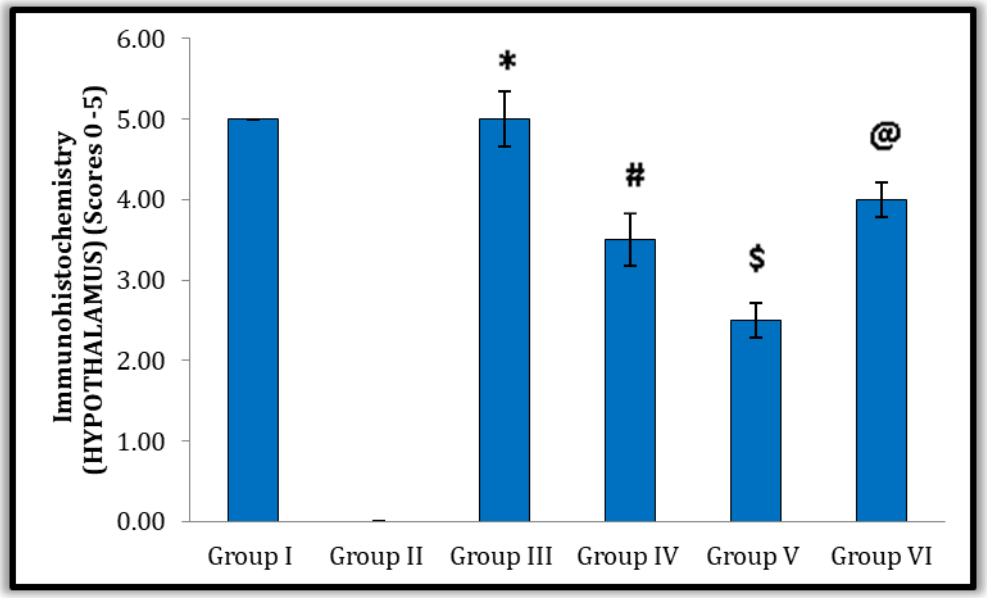


Figure 110: Bar diagram depicting the immunohistochemistry examination scores in the Hypothalamus in Rotenone Model screening test in wistar albino rats

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the immunohistochemistry scores in the hypothalamus were found to be lower in the negative control group [Group II], which was statistically significant (P<0.05). It was also revealed that the experimental groups [Group IV, Group V and Group VI] as well as the standard drug group [Group III] (Captopril, Perindopril and Losartan) that there was an increase in the IHC scores in the hypothalamus which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 17: Immunohistochemistry examination scores in Rotenone Model screening test in wistar albino rats

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0 -5)	5.00±0.17	0.00±0.17	4.00±0.37	3.50±0.22	3.50±0.49	4.00±0.26
2	Prefrontal Cortex (Scores 0 -5)	5.00±0.00	0.00±0.21	4.00±0.17	3.50±0.33	3.00±0.21	4.00±0.37
3	Striatum (Scores 0 -5)	5.00±0.00	0.00±0.17	4.00±0.26	3.00±0.48	3.00±0.48	4.00±0.31
4	Hypothalamus (Scores 0 -5)	5.00±0.00	0.00±0.00	5.00±0.34	3.50±0.33	2.50±0.22	4.00±0.21

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were lower in the negative control group [Group II], which was statistically significant (P<0.05). The standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increased IHC scores in the hippocampus, prefrontal cortex, corpus striatum and the hypothalamus and which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

II. MPTP MODEL IN SWISS ALBINO MICE

Evaluation of neurobehavioral activity

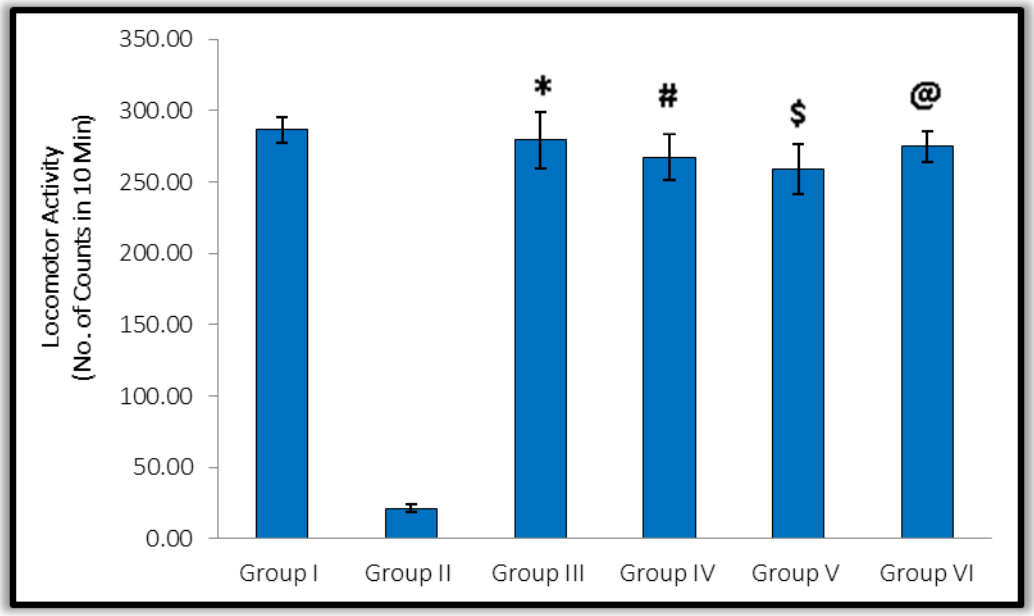


Figure 111: Bar diagram depicting the locomotor activity in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results revealed that the standard drug group [Group III] (Levodopa+Benserazide drug) had a substantial increase in the number of counts when compared to the negative control group [Group II] (P<0.05). Similarly, as compared to the negative control group [Group II], all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a rise in the total number of counts, which was statistically significant (P<0.05).

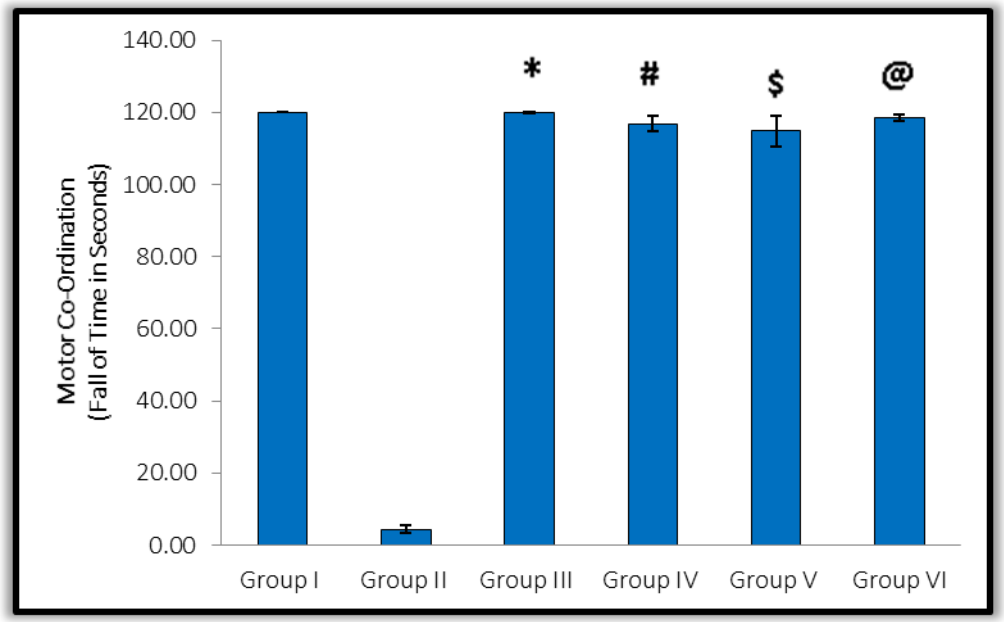


Figure 112: Bar diagram depicting the motor co-ordination [rotarod test] in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results showed that when comparing the standard drug group [Group III] (Levodopa+Benserazide drug) to the negative control group [Group II] (P<0.05), the standard group [Group III] (Levodopa+Benserazide drug) had a significant increase in the fall of time. When compared to the negative control group [Group II], all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) reported an increase in the fall of time that was statistically significant (P<0.05).

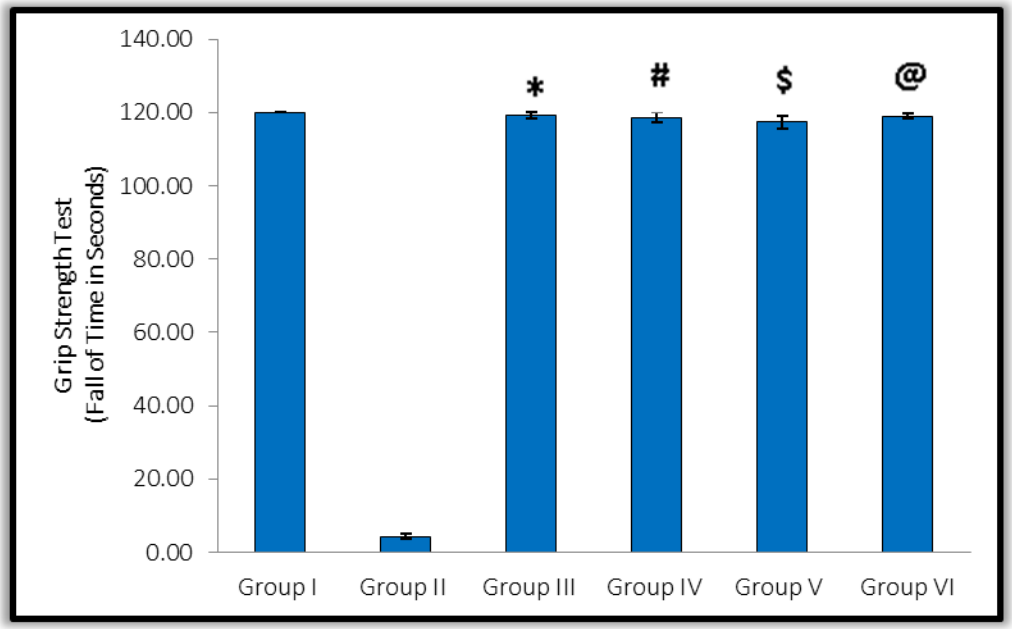


Figure 113: Bar diagram depicting the grip strength test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa + Benserazide group [Group III] (Standard drug group) to the negative control group [Group II] (P<0.05), there was a significant increase in the fall time of the Levodopa + Benserazide group [Group III] (Standard drug). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant (P<0.05) increase in the fall of time.

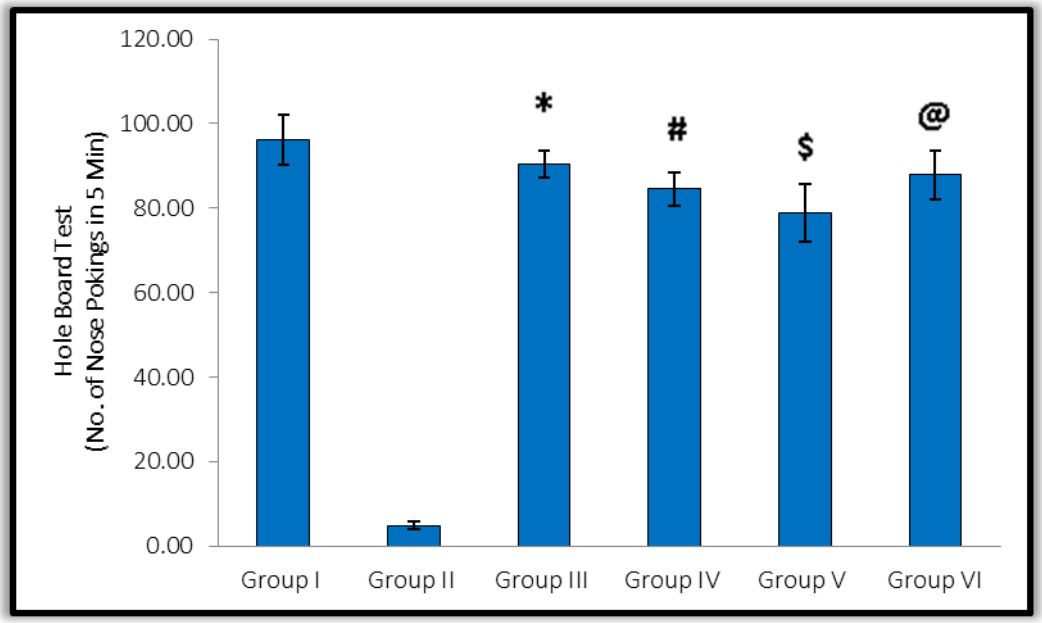


Figure 114: Bar diagram depicting the hole board test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The Levodopa+Benserazide group [Group III] (Standard drug group) had a significant increase in the parameter (Nose Poking in Hole Board Test) when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) found a statistically significant (P<0.05) increase in nose poking.

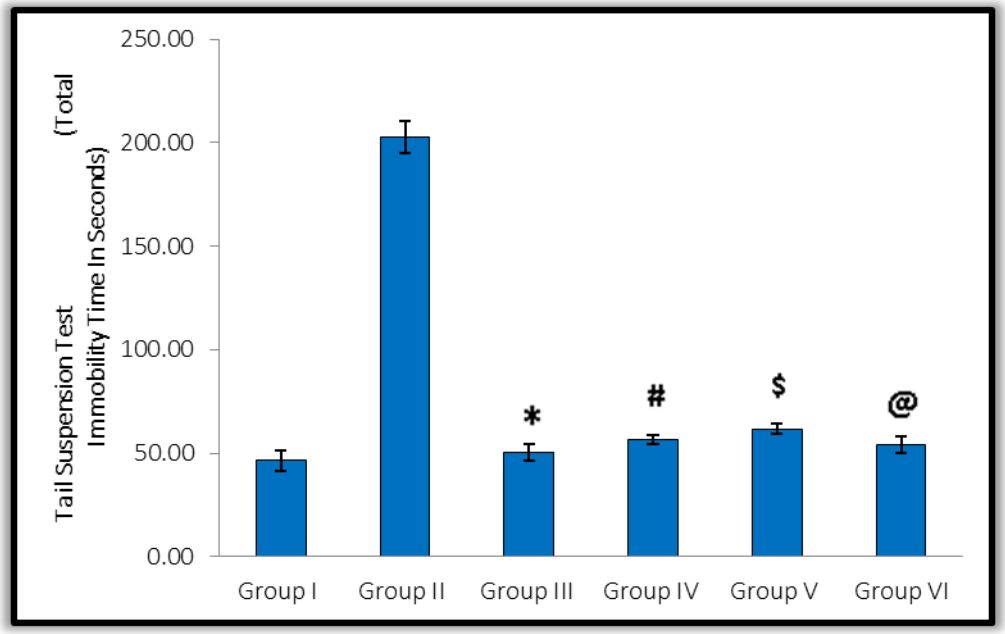


Figure 115: Bar diagram depicting the tail suspension test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

In the parameter (Total Immobility Time in Tail Suspension Test), the Levodopa+Benserazide group [Group III] (Standard drug group) showed a significant reduction when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) found a statistically significant (P<0.05) reduction in total immobility time.

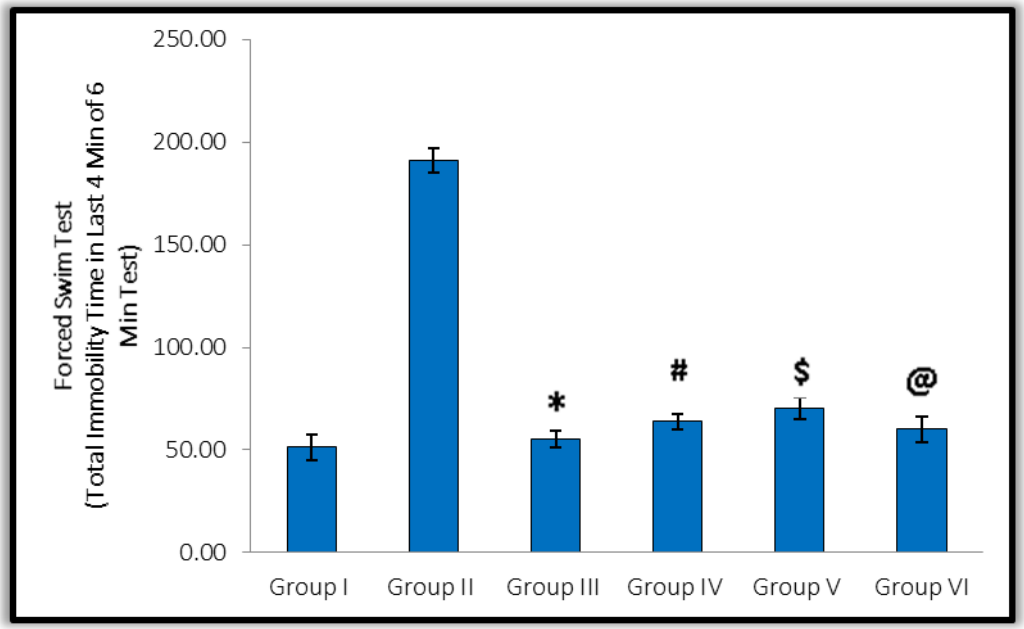


Figure116: Bar diagram depicting the forced swim test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Similarly, in the parameter (Total Immobility Time in Forced Swim Test), the Levodopa+Benserazide group [Group III] (Standard drug group) showed a significant decrease when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant (P<0.05) reduction in total immobility time.

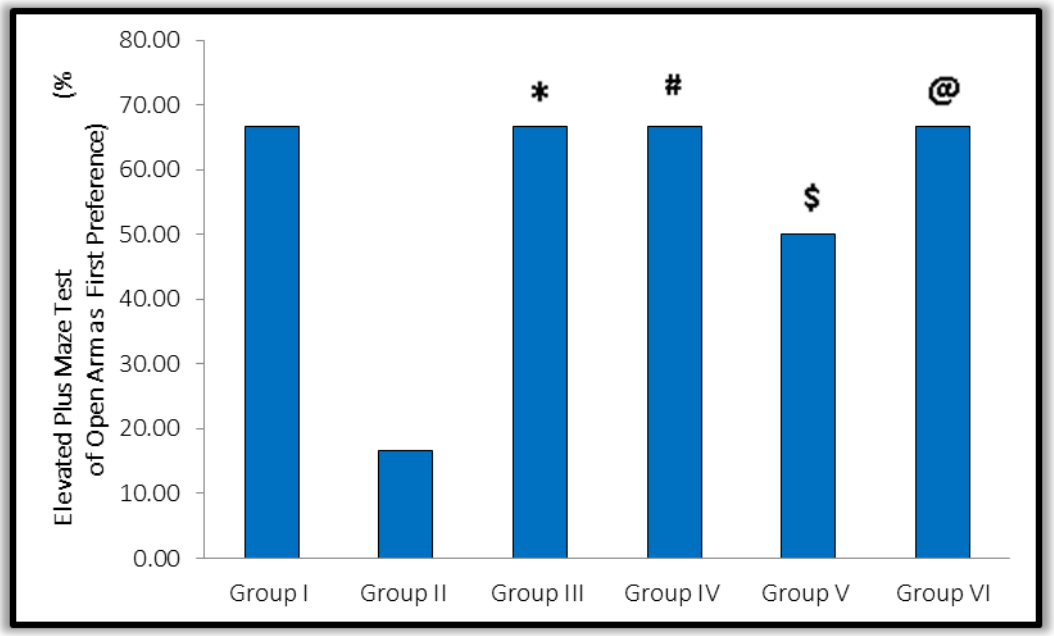


Figure 117: Bar diagram depicting the percentage of open arm as first preference in elevated plus maze test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group [Group III] (Standard drug group) to the negative control group [Group II] (P<0.05), there was a substantial increase in the percentage of open arm as the first arm preference in the Elevated Plus Maze Test. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant (P<0.05) increase in the percentage of open arms as first arm choice.

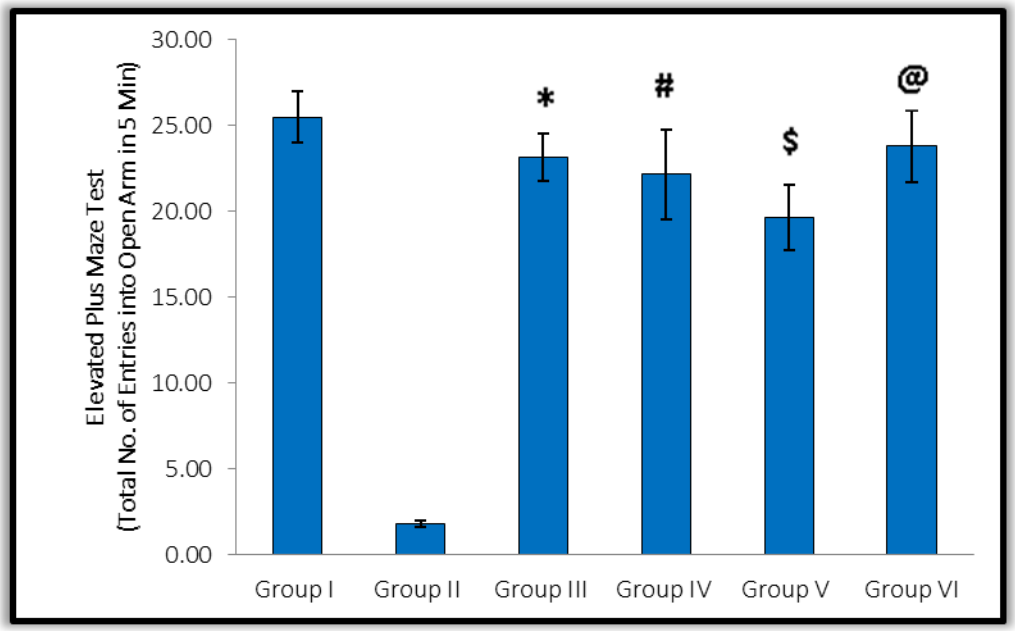


Figure 118: Bar diagram depicting the total number of entries into the open arm in elevated plus maze test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant increase in the Levodopa+Benserazide group [Group III] (Standard drug group) as compared to the negative control group [Group II] (P<0.05) in the parameter (Total Number of Entries into Open Arm in the Elevated Plus Maze Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistically significant (P<0.05) increase in the total number of entries into the open arm.

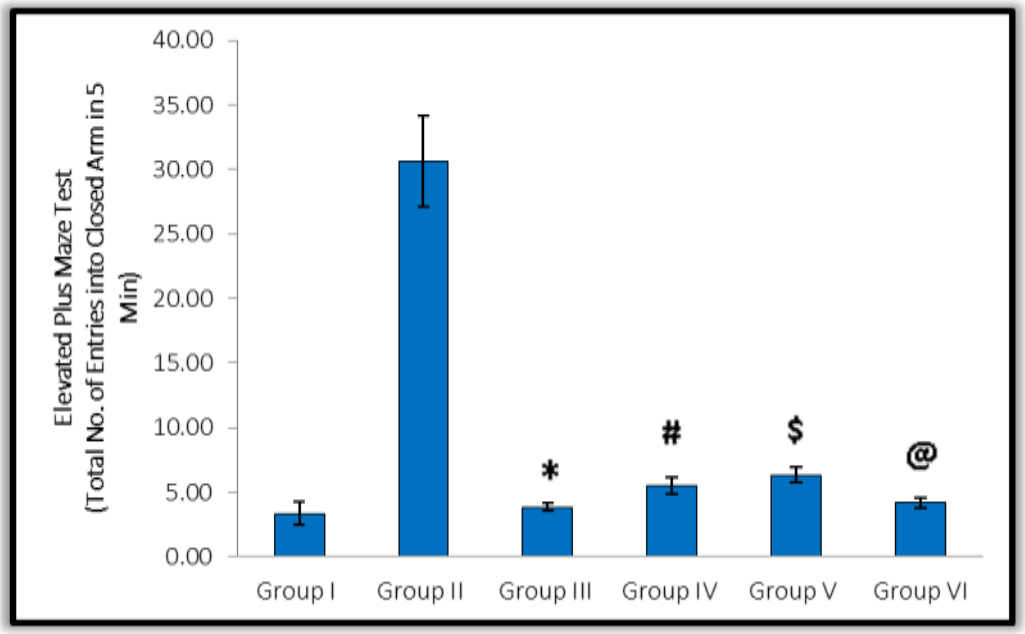


Figure 119: Bar diagram depicting the total number of entries into the closed arm in elevated plus maze test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant decrease in the Levodopa+Benserazide group [Group III] (Standard drug group) as compared to the negative control group [Group II] (P<0.05) in the parameter (Total Number of Entries into Closed Arm in Elevated Plus Maze Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) found a statistically significant (P<0.05) decline in the total number of entries into the closed arm.

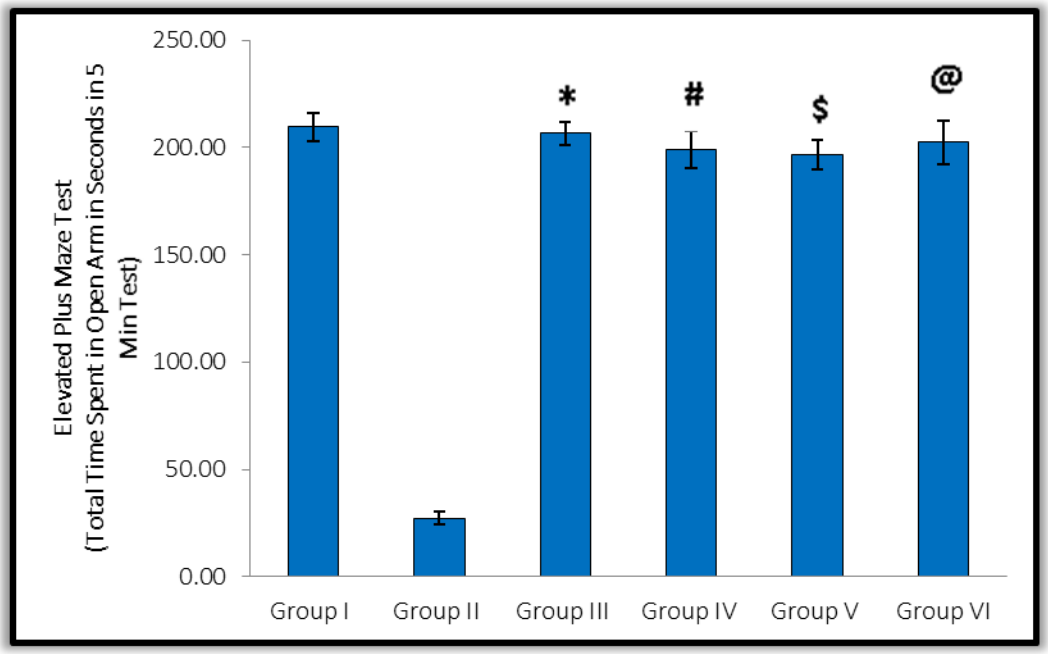


Figure 120: Bar diagram depicting the total time spent in the open arm in elevated plus maze test in Methyl Phenyl Tetrahydropyridine [MPTP] model screening test in wiss lbino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant increase in the Levodopa+Benserazide group [Group III] (Standard drug group) as compared to the negative control group [Group II] (P<0.05) in the parameter (Time Spent In Open Arm in Elevated Plus Maze Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant (P<0.05) increase in time spent in the open arm.

Table 18: Behavioural analysis in MPTP model

Group	Actophotometer test	Rotarod test	Grip strength test	Hole board test	Tail suspension test	Force swim test	Elevated plus maze			
							% Open arm preference	No. of entries into the open arm	No. of entries into the close arm	Time spent in the open arm
I [Vehicle control]	286.50±8.77	120.00±0.00	120.00±0.00	96.17±5.88	46.50±4.97	51.33±6.08	66.67	25.50±1.48	3.33±0.92	209.50±6.37
II [Negative control]	21.17±2.70	4.33±1.02	4.33±0.76	4.83±0.95	202.67±7.70	191.17±6.01	16.67	1.83±0.17	30.67±3.53	27.33±2.79
III [Standard control]	279.17±20.14	119.83±0.17	119.17±0.83	90.50±3.23	50.33±3.81	55.50±4.13	66.67	23.17±1.40	3.83±0.31	206.50±5.40
IV [Captopril]	267.33±16.01	116.83±2.04	118.67±1.33	84.50±3.87	56.50±2.40	63.83±3.96	66.67	22.17±2.63	5.50±0.67	199.17±8.63
V [Perindopril]	259.17±17.67	114.83±4.25	117.33±1.76	78.83±6.84	61.67±2.32	70.17±5.49	50.00	19.67±1.89	6.33±0.56	196.67±7.07
VI [Losartan]	274.67±11.15	118.50±0.96	119.00±0.68	87.83±5.91	53.83±4.00	60.17±6.23	66.67	23.83±2.09	4.17±0.40	202.50±10.17

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Evaluation of oxidative stress markers

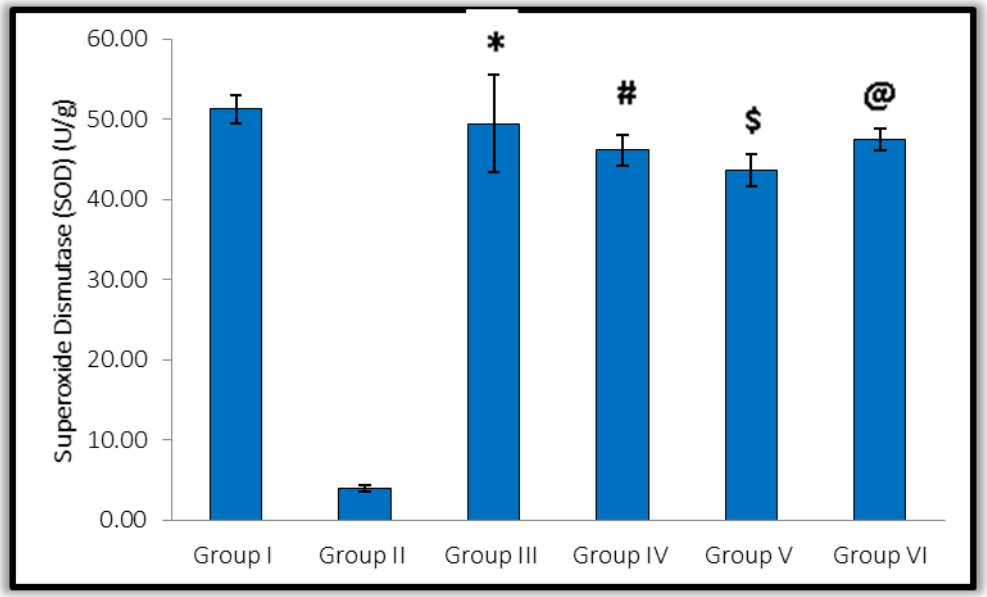


Figure 121: Bar diagram depicting the anti-oxidant enzyme (*Superoxide dismutase*) levels in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Superoxide dismutase) level was significantly (P<0.05) lower in the negative control group [Group II]. The study found that when compared to the negative control group [Group II], the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a raised level of the anti-oxidant enzyme (Superoxide dismutase), which was statistically significant (P<0.05).

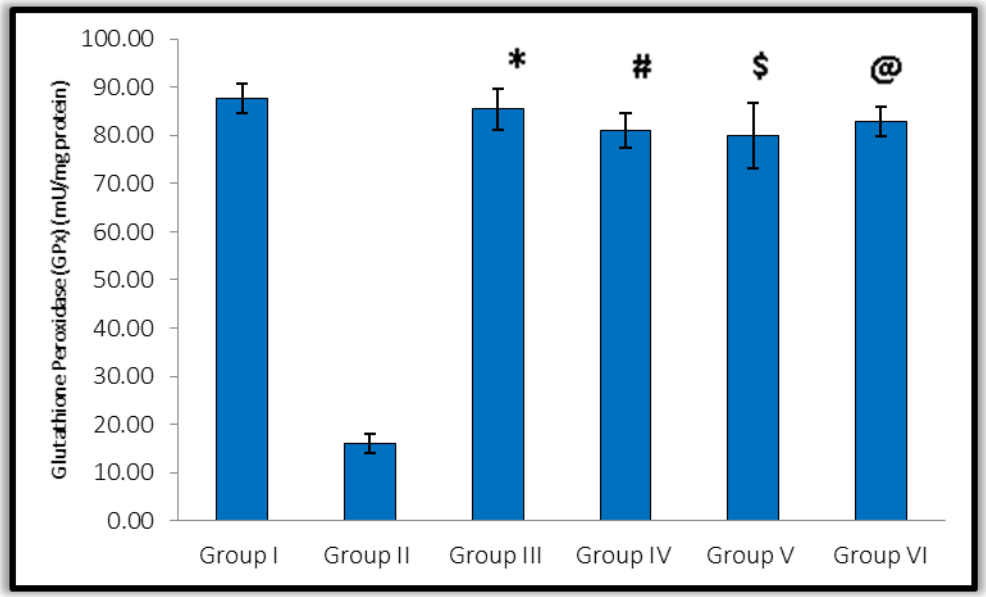


Figure 122: Bar diagram depicting the anti-oxidant enzyme (*Glutathione peroxidase*) level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Similarly, when compared to the vehicle control group [Group I], the anti-oxidant enzyme (glutathione peroxidase) level was substantially (P<0.05) lower in the negative control group [Group II]. The study also discovered that the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI]

(Captopril, Perindopril and Losartan) had increased anti-oxidant enzyme (*Glutathione peroxidase*) levels and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

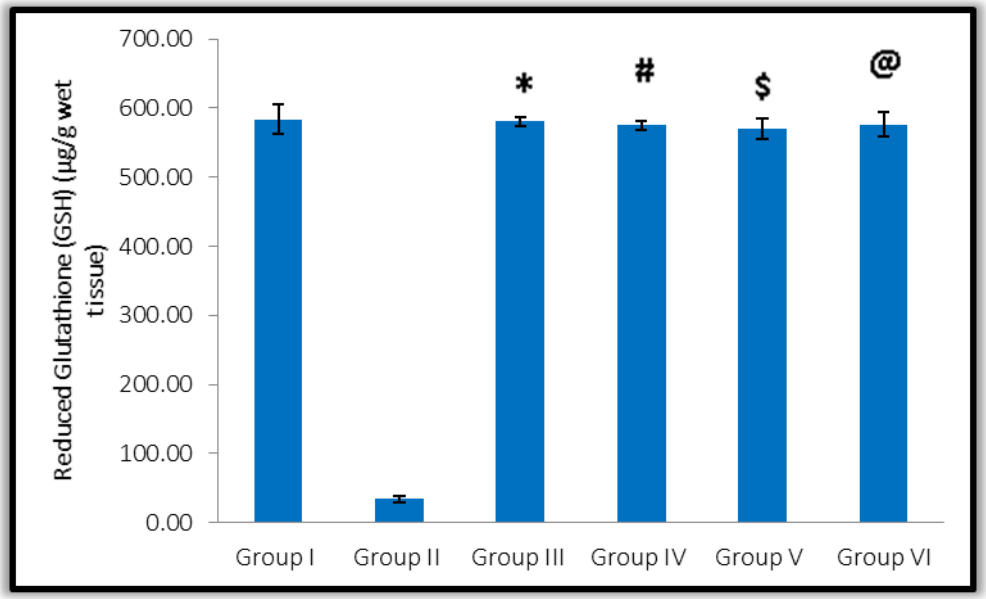


Figure 123: Bar diagram depicting the anti-oxidant enzyme (*Reduced glutathione*) level in in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (reduced glutathione) levels in the negative control group [Group II] was decreased (P<0.05). The study also depicted that when compared to the negative control group [Group II], the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increased level of anti-oxidant enzyme (reduced glutathione) levels, which was statistically significant (P<0.05).

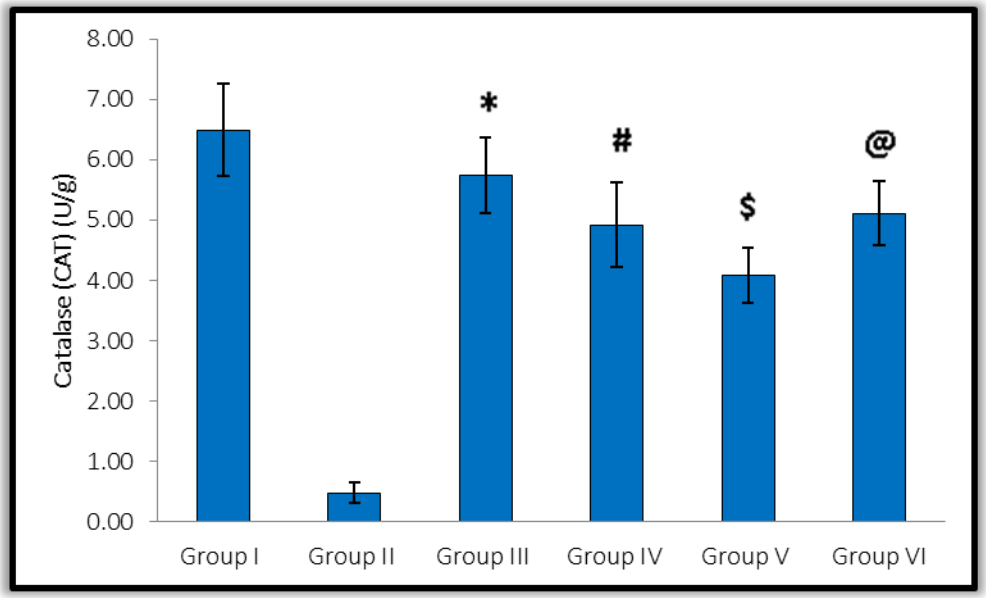


Figure 124: Bar diagram depicting the anti-oxidant enzyme (*Catalase*) level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\$}P < 0.05$ and $^{@}P < 0.05$, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Catalase) level in the negative control group [Group II] was decreased ($P<0.05$). The study also found that when compared to the negative control group [Group II], the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increased level of the anti-oxidant enzyme (Catalase), and this was statistically significant ($P<0.05$).

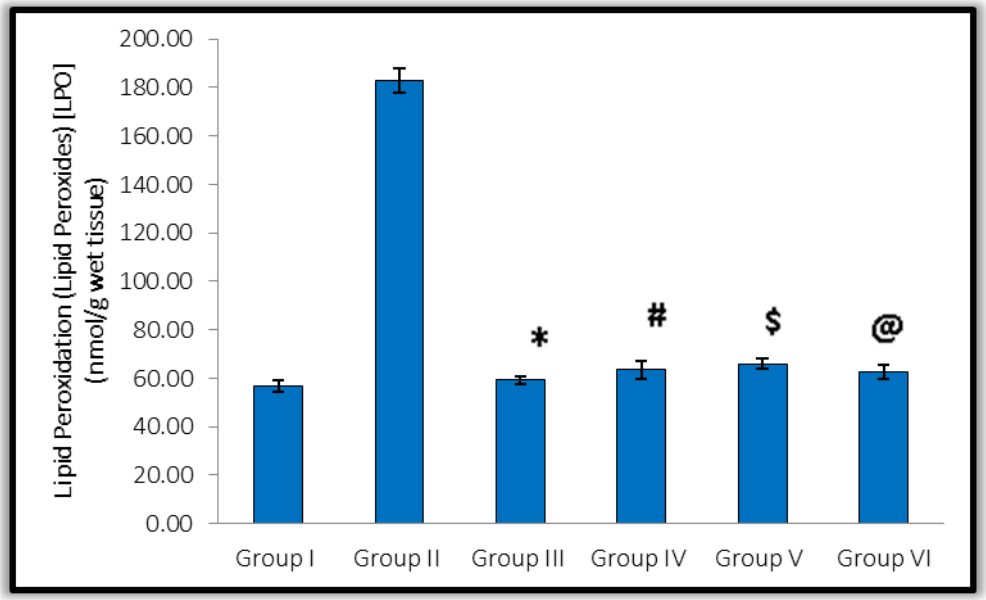


Figure 125: Bar diagram depicting the *lipid peroxidation* level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], lipid peroxidation was considerably higher in the negative control group [Group II]. The study also revealed that when compared to the negative control group [Group II], the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) had a lower degree of lipid peroxidation, which was statistically significant (P<0.05).

Table 19: Anti-oxidant enzymes in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Superoxide Dismutase (SOD) (*U/g*)	51.23±1.72	3.98±0.43	49.40±6.07	46.11±1.95	43.62±2.02	47.39±1.36
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	87.62±3.09	16.16±2.03	85.46±4.26	81.05±3.56	79.84±6.81	82.87±3.09
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	583.65±21.29	34.21±4.81	580.83±6.63	574.79±7.07	570.22±15.63	576.52±17.70
4	Catalase (CAT) (*U/g*)	6.49±0.76	0.47±0.17	5.74±0.63	4.92±0.71	4.08±0.45	5.11±0.53
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	56.65±2.54	182.85±4.98	59.24±1.60	63.41±3.69	65.98±2.03	62.39±2.93

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], all anti-oxidant enzyme levels (superoxide dismutase, glutathione peroxidase, reduced glutathione and catalase) were significantly lower in the negative control group [Group II] (P<0.05). The study also indicated that there was an increased level of anti-oxidant enzymes (superoxide dismutase, glutathione peroxidase, reduced glutathione and catalase) in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) that was statistically significant when compared to the negative control group [Group II] (P<0.05).

Evaluation of neurotransmitters

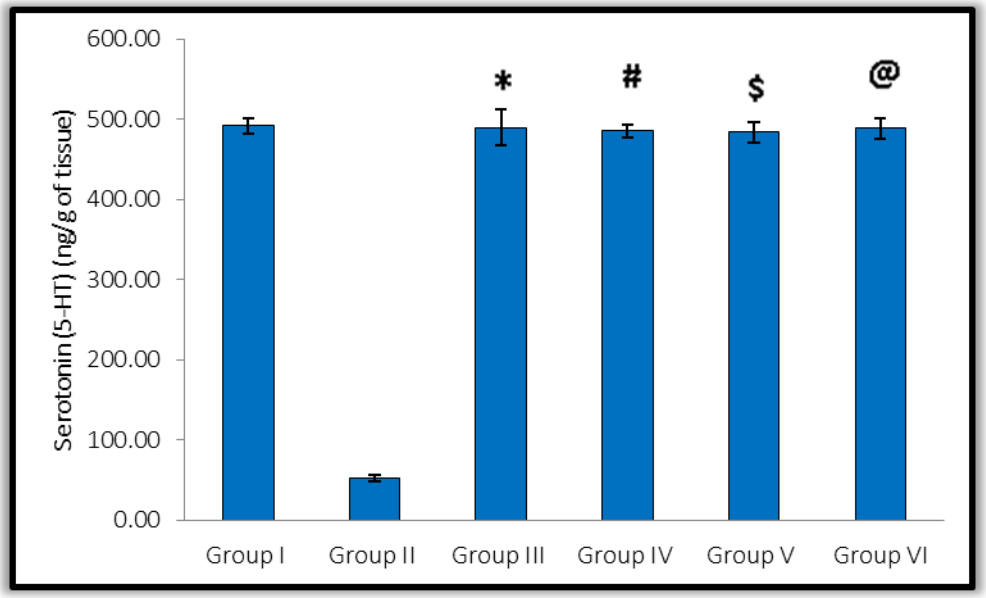


Figure 126: Bar diagram depicting the serotonin level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (Serotonin) level was decreased in the negative control group [Group II], which was statistically significant (P<0.05). There was also a statistically significant rise in the neurotransmitter (Serotonin) level in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as compared to the negative control group [Group II] (P<0.05).

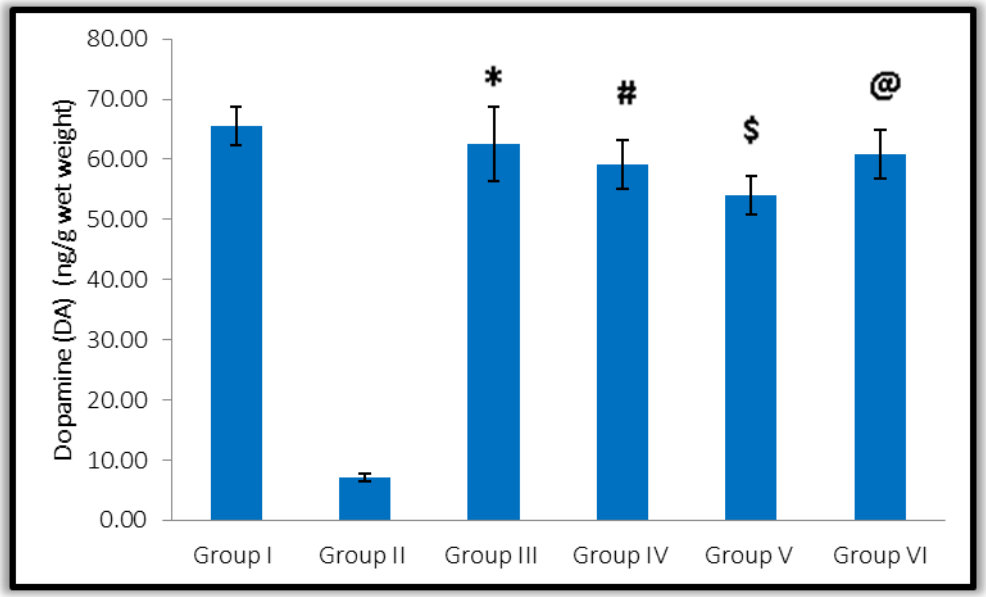


Figure 127: Bar diagram depicting the *Dopamine* level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (Dopamine) level was declined in the negative control group [Group II], which was statistically significant (P<0.05). There was also a statistically significant increase in the neurotransmitter (Dopamine) levels in the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) when compared to the negative control group [Group II] (P<0.05).

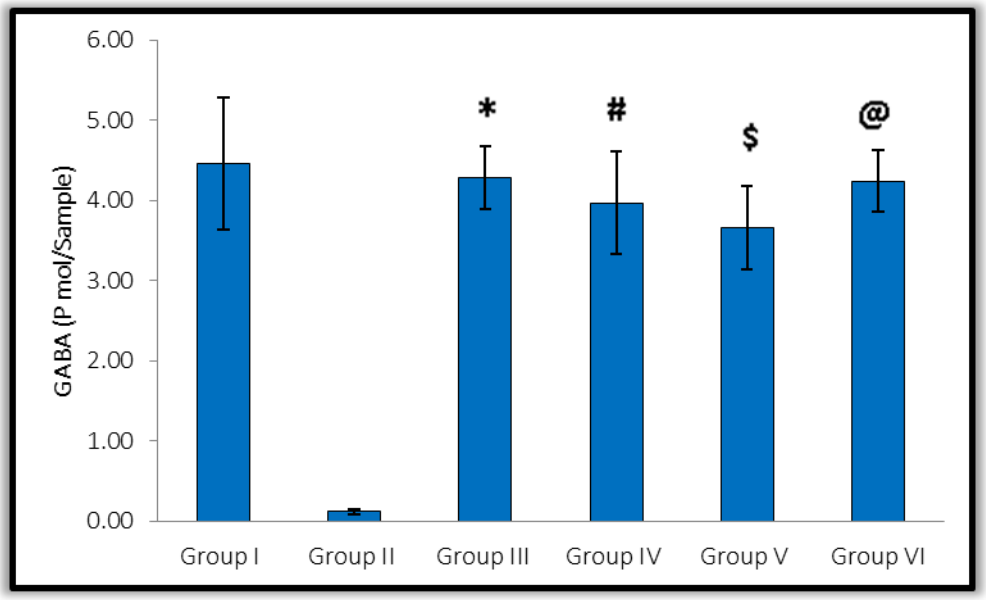


Figure 128: Bar diagram depicting the *GABA* level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (GABA) level was reduced in the negative control group [Group II], which was statistically significant (P<0.05). There was a statistically significant rise in the neurotransmitter (GABA) level in the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as compared to the negative control group [Group II] (P<0.05).

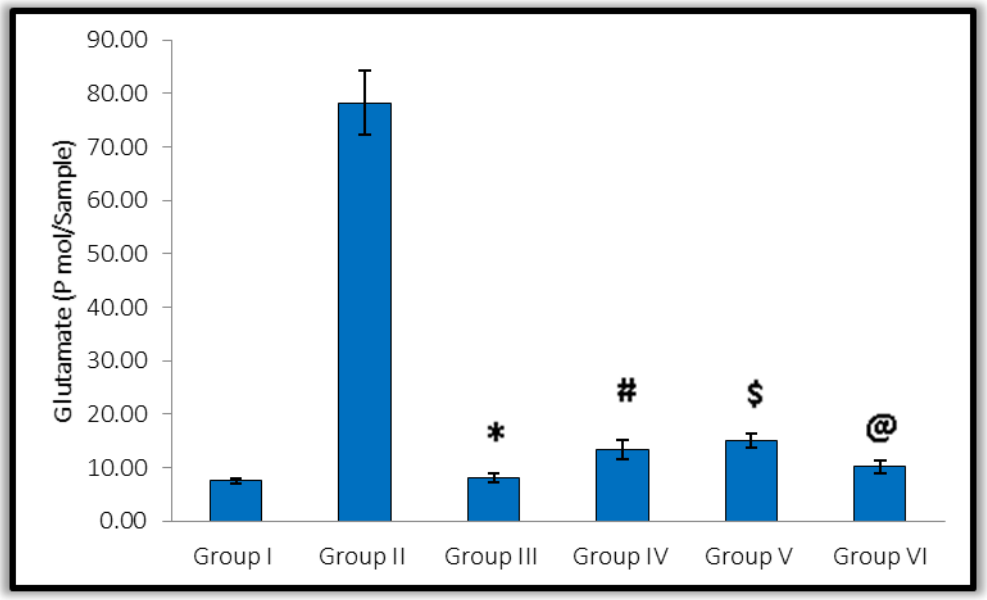


Figure 129: Bar diagram depicting the *Glutamate* level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (glutamate) level was found to be higher in the negative control group [Group II], which was statistically significant (P<0.05). There was a statistically significant decrease in the neurotransmitter (Glutamate) level in the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) when compared to the negative control group [Group II] (P<0.05).

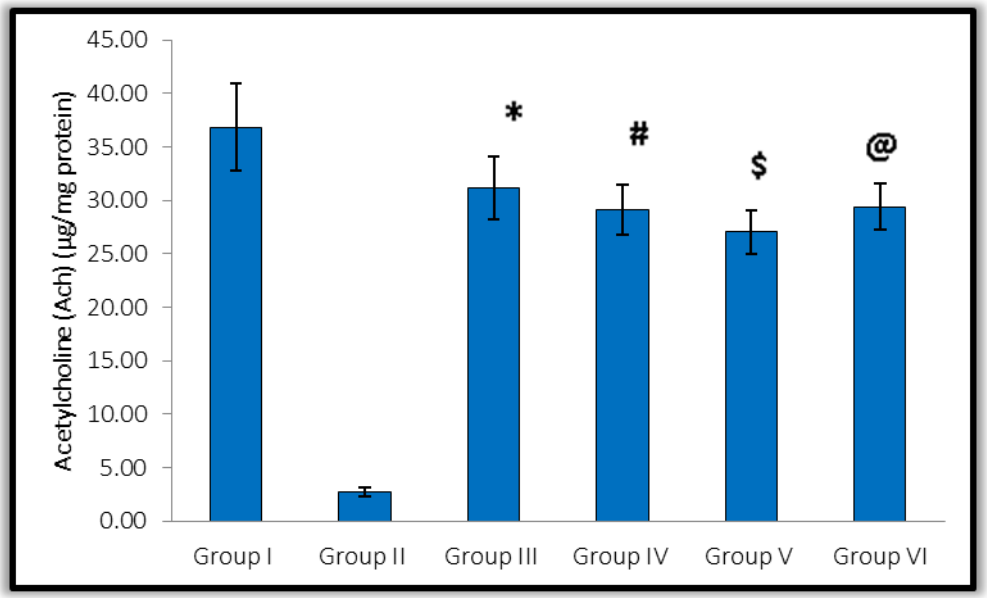


Figure 130: Bar diagram depicting the *Acetylcholine* level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (ACh) level was found to be decreased in the negative control group [Group II], which was statistically significant (P<0.05). It was also observed that there was a statistically significant increase in the neurotransmitter (ACh) level in the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) when compared to the negative control group [Group II] (P<0.05).

Table 20: Neurotransmitters in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Serotonin (*ng/g of tissue*)	491.84±9.82	51.85±3.97	489.29±22.46	485.51±8.04	483.72±12.39	488.35±13.38
2	Dopamine (*ng/g wet weight*)	65.50±3.23	7.08±0.69	62.58±6.20	59.15±4.13	54.07±3.23	60.91±4.04
3	GABA (*P mol/Sample*)	4.46±0.83	0.12±0.03	4.28±0.39	3.97±0.64	3.66±0.52	4.24±0.38
4	Glutamate (*P mol/Sample*)	7.53±0.45	78.27±6.09	8.13±0.94	13.41±1.71	15.09±1.34	10.18±1.25
5	Acetylcholine (*µg/mg protein*)	36.85±4.03	2.77±0.44	31.21±2.95	29.09±2.36	27.05±2.01	29.41±2.16

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the neurotransmitter (5-HT, DA, GABA and ACh) levels were decreased in the negative control group [Group II], which was statistically significant (P<0.05). There was also a statistically significant rise in the neurotransmitter (5-HT, DA, GABA and ACh) levels in the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as compared to the negative control group [Group II] (P<0.05).

When comparing the negative control group [Group II] to the vehicle control group [Group I], the neurotransmitter (Glutamate) level was shown to be increased significantly (P<0.05) in the negative control group. There was a decrease in the neurotransmitter (Glutamate) level in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Evaluation of inflammatory marker - Myeloperoxidase [MPO]

Table 21: Evaluation of inflammatory marker [MPO] in MPTP model

Group	I [Vehicle control]	II [Negative control]	III [Standard control]	IV [Captopril]	V [Perindopril]	VI [Losartan]
MPO	0.53±0.11	8.93±0.79	0.89±0.08	1.23±0.10	1.51±0.24	1.08±0.19

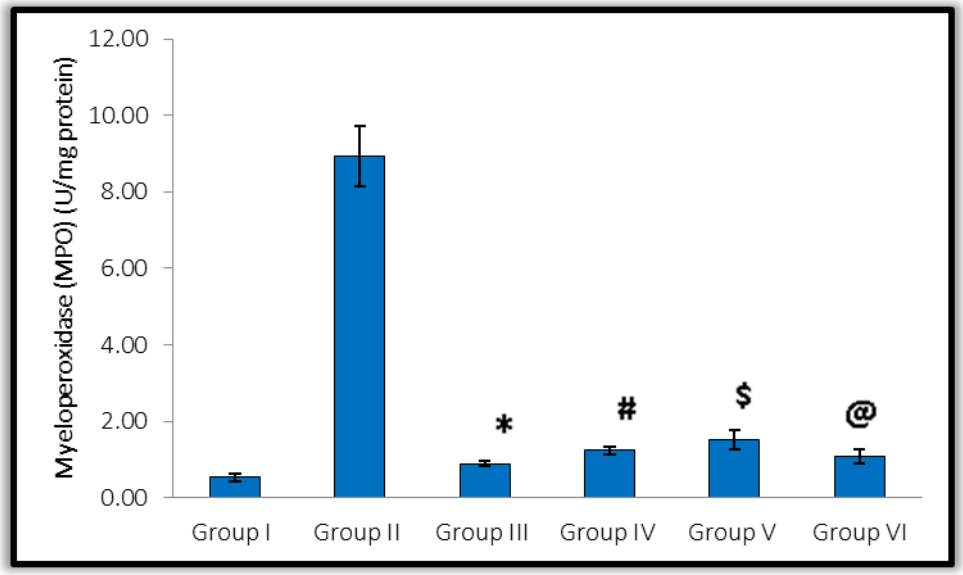


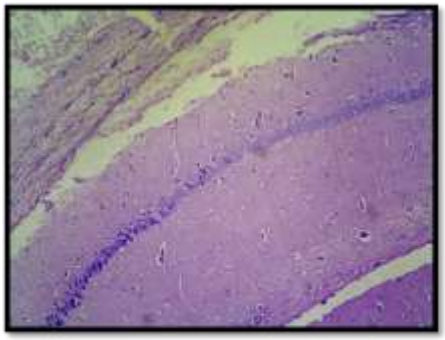
Figure 131: Bar diagram depicting the inflammatory marker (*Myeloperoxidase*) level in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had a higher level of myeloperoxidase, which was statistically significant (P<0.05). There was also a drop in the Myeloperoxidase level in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

MICROANATOMICAL [HISTOPATHOLOGICAL] STUDY IN MPTP MODEL

Microanatomical [Histopathological] study of Hippocampus in MPTP model

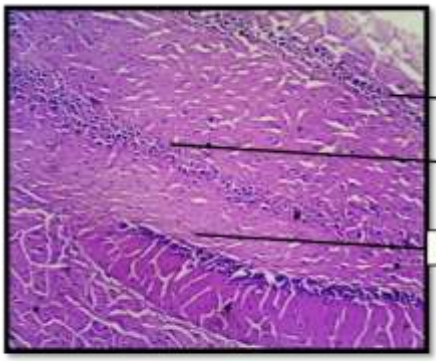


Section studied from the mouse brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 132: Section of mouse brain [**Hippocampus**] from **Vehicle control group [Group I]** in **MPTP model**

(10x; H & E stained)

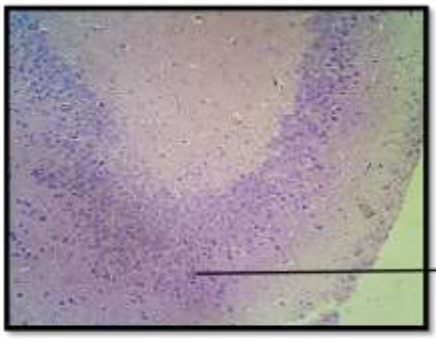


Section studied from the mouse brain [Hippocampus] of MPTP alone group [Group II] showing severe sclerosis, gliosis and degeneration amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 133 : Section of mouse brain [**Hippocampus**] from **MPTP alone group [Group II]** in **MPTP model**

(10x; H & E stained)

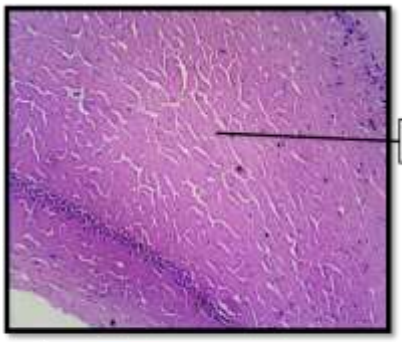


Section studied from the mouse brain [Hippocampus] of MPTP + Positive control group [Group III] showing mild gliosis and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 134: Section of mouse brain [**Hippocampus**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model**

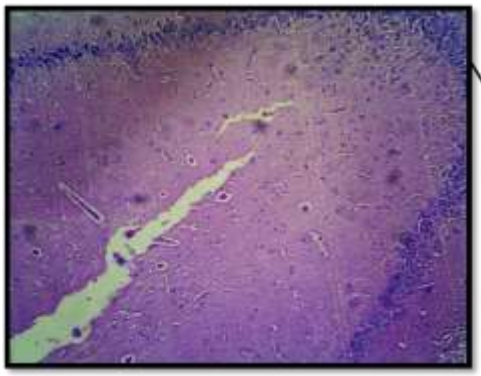
(10x; H & E stained)



Section studied from the mouse brain [Hippocampus] of MPTP + Captopril group [Group IV] showing moderate degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

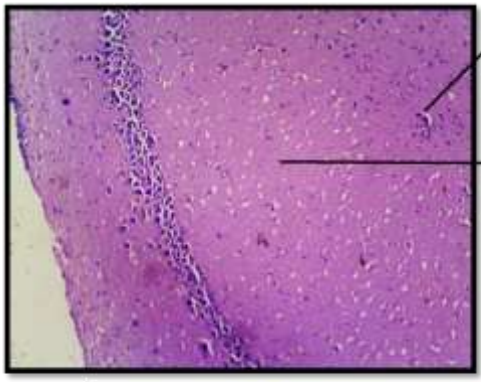
Figure 135: Section of mouse brain [**Hippocampus**] from **MPTP + Captopril [Group IV]** in **MPTP model**
(10x; H & E stained)



Section studied from the mouse brain [Hippocampus] of MPTP + Perindopril group [Group V] showing mild sclerosis and degeneration amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 136: Section of mouse brain [**Hippocampus**] from **MPTP + Perindopril [Group V]** in **MPTP model**
(10x; H & E stained)



Section studied from the mouse brain [Hippocampus] of MPTP + Losartan group [Group VI] showing mild gliosis and vacoulations amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 137: Section of mouse brain [**Hippocampus**] from **MPTP + Losartan [Group VI]** in **MPTP model**
(10x; H & E stained)

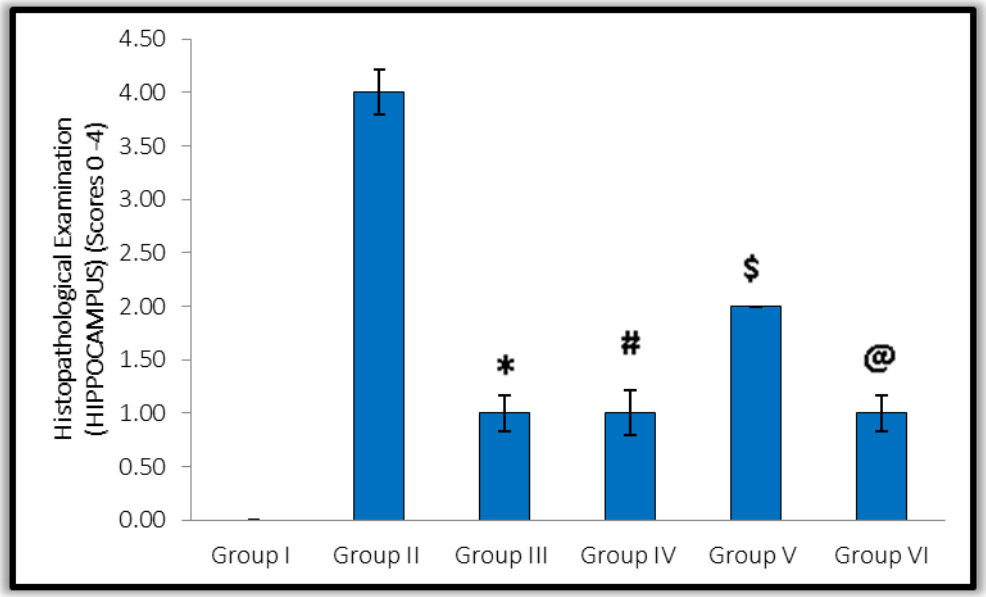
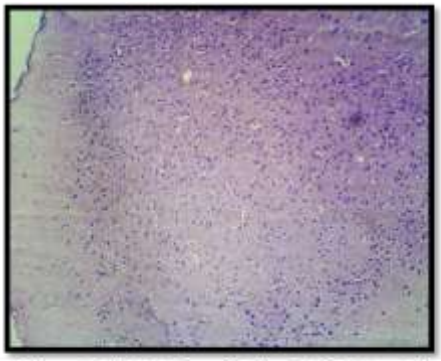


Figure 138: Bar diagram depicting the histopathological examination scores in the Hippocampus in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hippocampus, which was statistically significant (P<0.05). It was also found that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a decreased level in the histopathological scores in hippocampus, and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

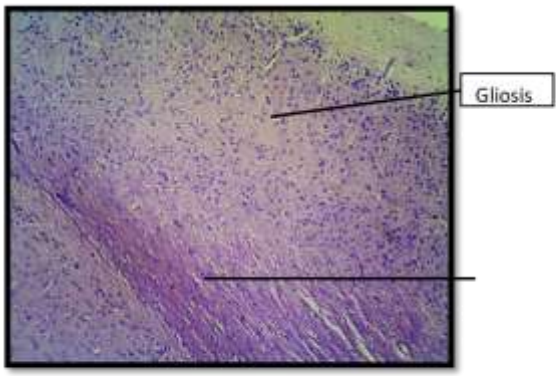
Microanatomical [Histopathological] study of Prefrontal cortex [Cerebrum] in MPTP model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

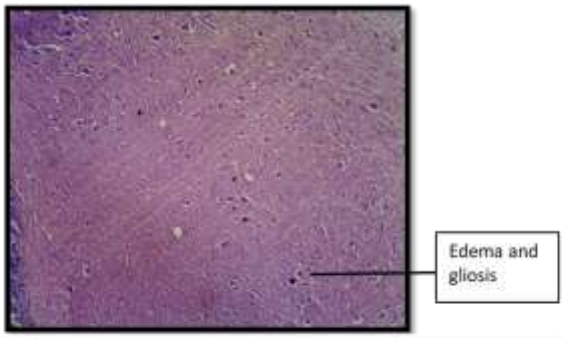
Figure 139: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **MPTP model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MPTP alone group [Group II] showing dense gliosis and inflammation amounting to the histopathological score 4.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

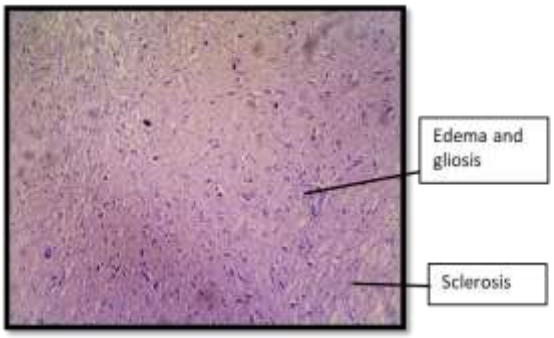
Figure 140: Section of rat brain [**Prefrontal cortex**] from **MPTP alone group [Group II]** in **MPTP model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MPTP + Positive control group [Group III] showing mild edema and gliosis amounting to the histopathological score 1.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 141 : Section of rat brain [**Prefrontal cortex**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (10x; H & E stained)

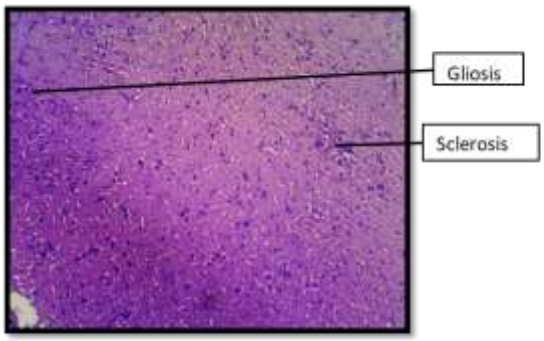


Section studied from the rat brain [Prefrontal cortex] of MPTP + Captopril group [Group IV] showing moderate sclerosis, edema and gliosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 142 : Section of rat brain [**Prefrontal cortex**] from **MPTP + Captopril [Group IV]** in **MPTP model**

(10x; H and E stained)

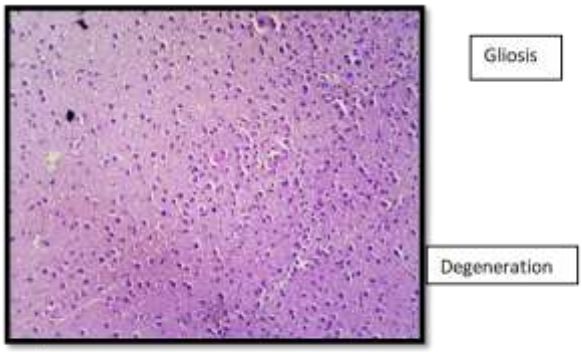


Section studied from the rat brain [Prefrontal cortex] of MPTP + Perindopril group [Group V] showing moderate sclerosis and gliosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 143: Section of rat brain [**Prefrontal cortex**] from **MPTP + Perindopril [Group V]** in **MPTP model**

(10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of MPTP + Losartan group [Group VI] showing mild gliosis and degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 144: Section of rat brain [**Prefrontal cortex**] from **MPTP + Losartan [Group VI]** in **MPTP model**

(10x; H & E stained)

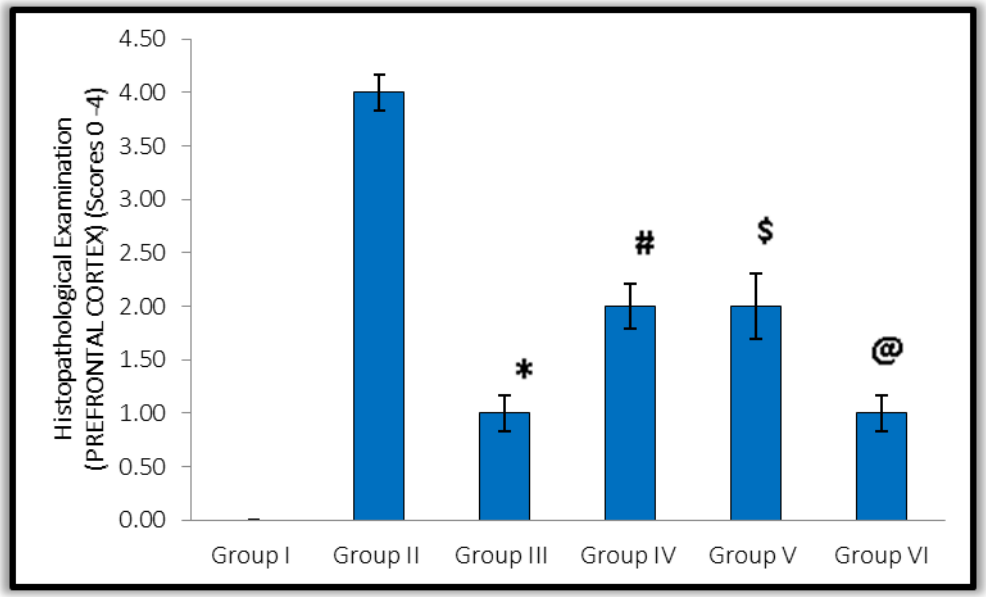
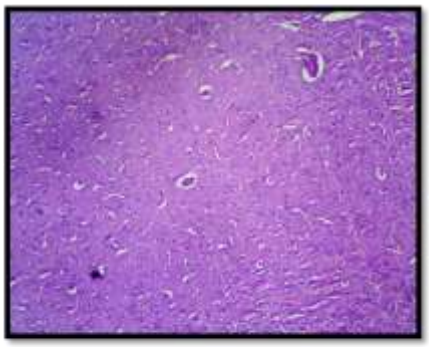


Figure 145: Bar diagram depicting the histopathological examination scores in the Prefrontal cortex in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the prefrontal cortex, which was statistically significant (P<0.05). It was also noticed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a decreased level in the histopathological scores in the prefrontal cortex which was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

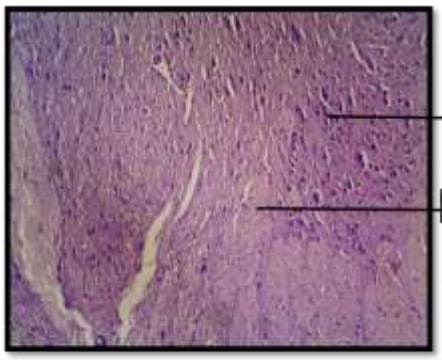
Microanatomical [Histopathological] study of Corpus striatum [Basal nuclei] in MPTP model



Section studied from the mouse brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

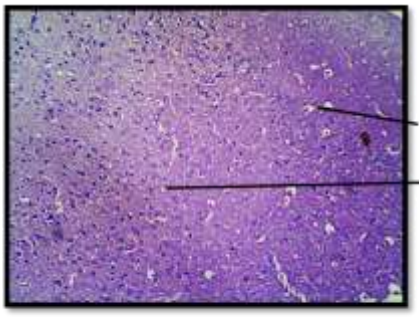
Figure 146: Section of mouse brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **MPTP Model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of MPTP alone group [Group II] showing severe gliosis and degeneration amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

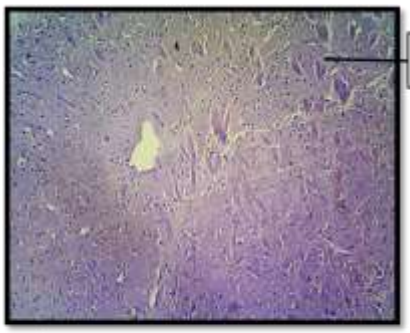
Figure 147: Section of mouse brain [**Corpus striatum**] from **MPTP alone group [Group II]** in **MPTP Model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of MPTP + Positive control group [Group III] showing mild vacoulations and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

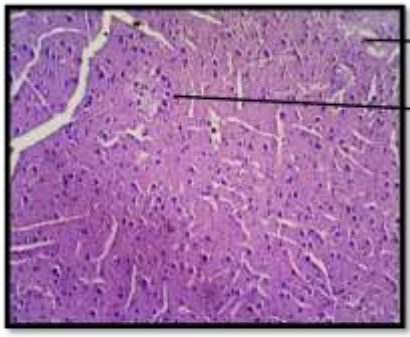
Figure 148: Section of mouse brain [**Corpus striatum**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP Model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of MPTP + Captopril group [Group IV] showing Sclerosis & gliosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

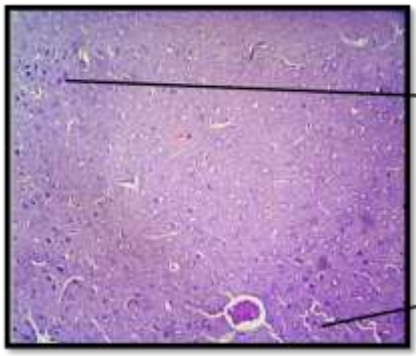
Figure 149: Section of mouse brain [**Corpus striatum**] from **MPTP + Captopril [Group IV]** in **MPTP Model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of MPTP + Perindopril group [Group V] showing moderate gliosis, degeneration and edema amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 150: Section of mouse brain [**Corpus striatum**] from **MPTP + Perindopril [Group V] in MPTP model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of MPTP +Losartan group [Group VI] showing mild gliosis and degeneration amounting to the histopathological score 1.5*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 151: Section of mouse brain [**Corpus striatum**] from **MPTP +Losartan [Group VI] in MPTP model** (10x; H & E stained)

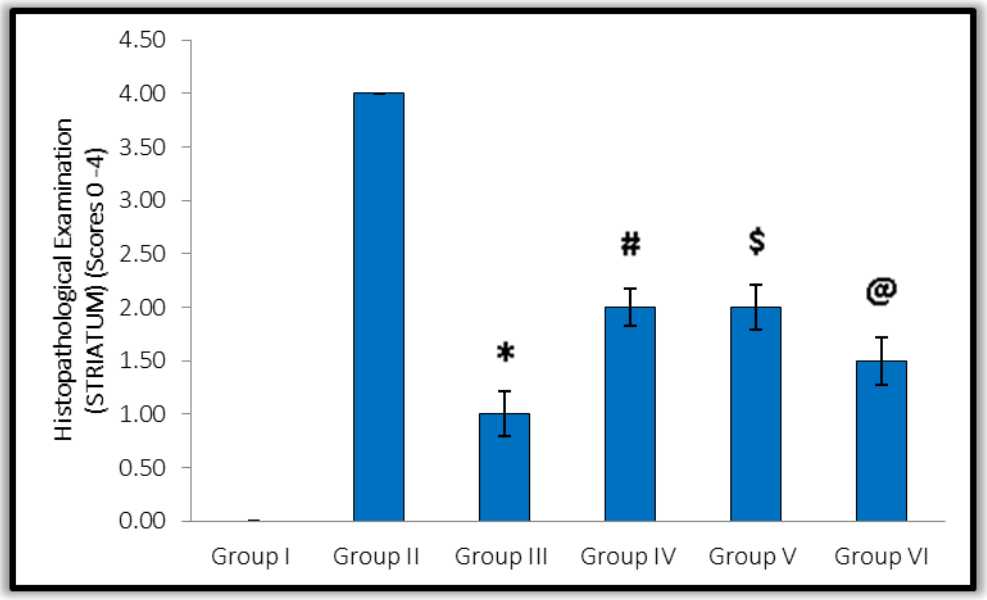
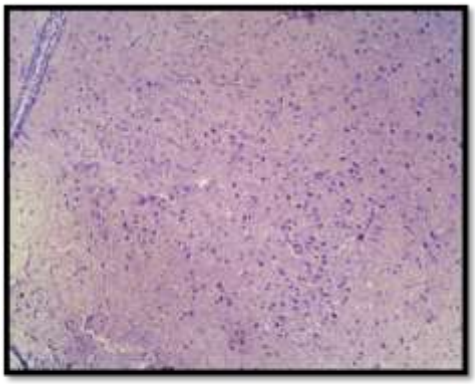


Figure 152: Bar diagram depicting the histopathological examination scores in the Corpus striatum in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the corpus striatum, which was statistically significant (P<0.05). There was also a decrease in the histopathological scores in the corpus striatum in the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

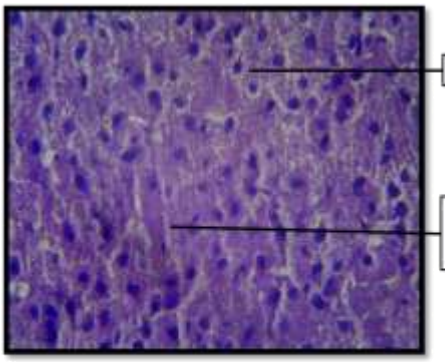
Microanatomical [Histopathological] study of Hypothalamus in MPTP model



Section studied from the mouse brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

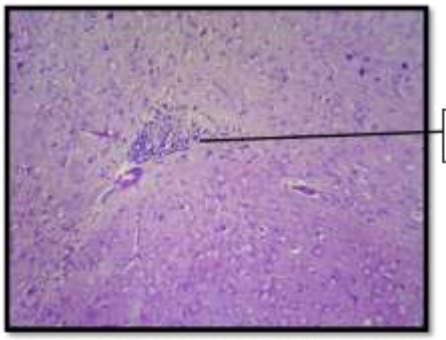
Figure 153: Section of mouse brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **MPTPmodel** (10x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of MPTP alone group [Group II] showing severe sclerosis amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

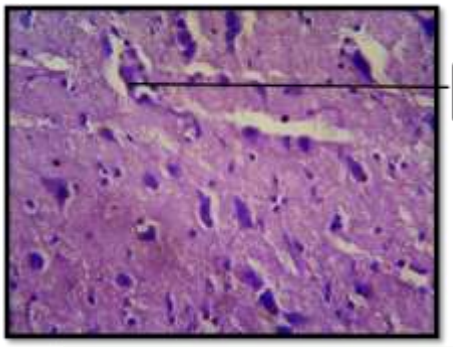
Figure 154: Section of mouse brain [**Hypothalamus**] from MPTP **alone group [Group II]** in **MPTP model** (40x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of MPTP + Positive control group [Group III] showing mild lymphatic infiltrates amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 155: Section of mouse brain [**Hypothalamus**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (10x; H & E stained)

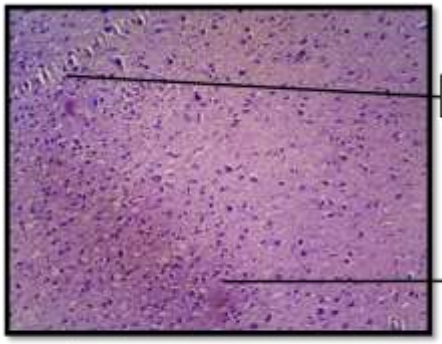


Section studied from the mouse brain [Hypothalamus] of MPTP + Captopril group [Group IV] showing moderate degeneration & edema amounting to the histopathological score 1.50*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 156: Section of mouse brain [**Hypothalamus**] from **MPTP + Captopril [Group IV]** in **MPTP model**

(40x; H & E stained)

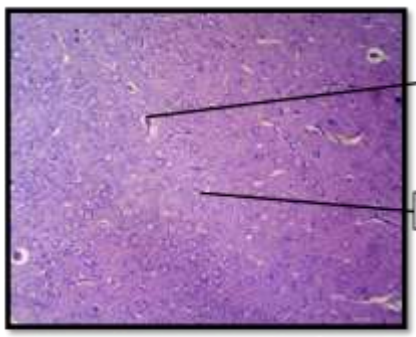


Section studied from the mouse brain [Hypothalamus] of MPTP + Perindopril group [Group V] showing moderate degeneration and gliosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 157: Section of mouse brain [**Hypothalamus**] from **MPTP + Perindopril [Group V]** in **MPTP model**

(10x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of MPTP +Losartan group [Group VI] showing pyknotic cells and degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells*, **1**: *>75% Healthy neuronal cells*, **2**: *50 - 74% Healthy neuronal cells*, **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 158: Section of mouse brain [**Hypothalamus**] **MPTP + Losartan [Group VI]** in **MPTP model**

(10x; H & E stained)

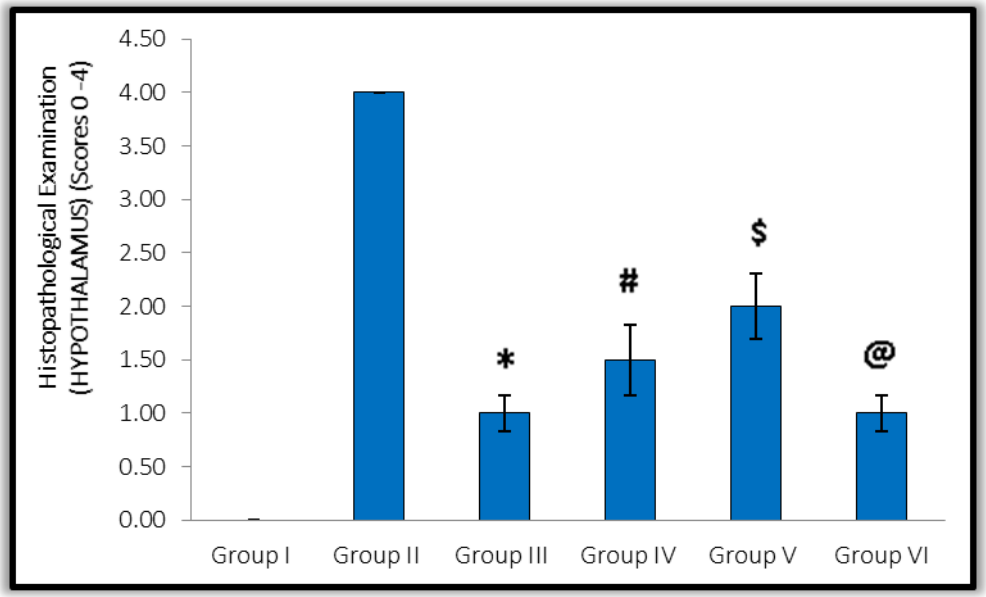


Figure 159: Bar diagram depicting the histopathological examination scores in the Hypothalamus in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hypothalamus, which was statistically significant (P<0.05). There was a decrease in the histopathological scores in the hypothalamus in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 22: Histopathological examination scores in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

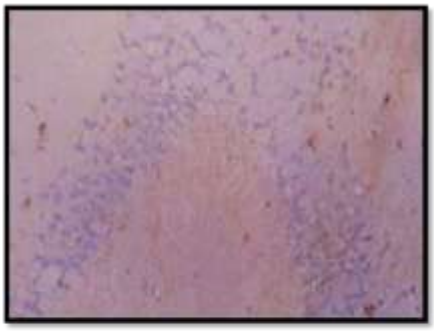
Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0 -4)	0.00±0.00	4.00±0.21	1.00±0.17	1.00±0.21	2.00±0.00	1.00±0.17
2	Prefrontal Cortex (Scores 0 -4)	0.00±0.00	4.00±0.17	1.00±0.17	2.00±0.21	2.00±0.31	1.00±0.17
3	Corpus striatum (Scores 0 -4)	0.00±0.00	4.00±0.00	1.00±0.21	2.00±0.17	2.00±0.21	1.50±0.22
4	Hypothalamus (Scores 0 -4)	0.00±0.00	4.00±0.00	1.00±0.17	1.50±0.33	2.00±0.31	1.00±0.17

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the negative control group [Group II] had higher histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus, which was statistically significant (P<0.05). There was also a decrease in the histopathological scores in the hippocampus, prefrontal cortex, striatum, and hypothalamus in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

EVALUATION OF IMMUNOHISTOCHEMISTRY IN MPTP MODEL

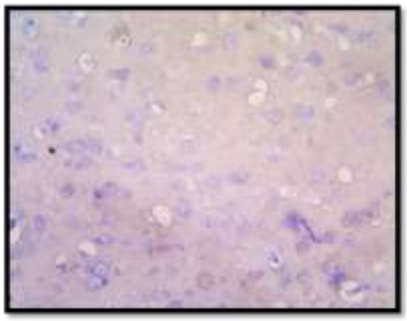
Immunohistochemistry of Hippocampus in various groups of MPTP model



Section studied from the mouse brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

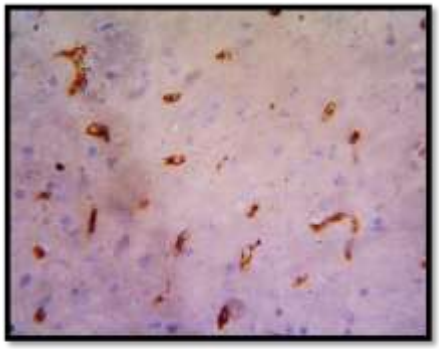
Figure 160: Section of mouse brain [**Hippocampus**] from **Vehicle control group [Group I]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] of MPTP alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0***:Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

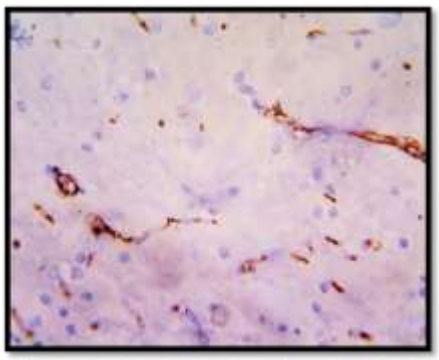
Figure 161: Section of mouse brain [**Hippocampus**] from **MPTP alone group [Group II]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] of MPTP + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 162: Section of mouse mouse brain [**Hippocampus**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (40x; IHC Bcl-2)

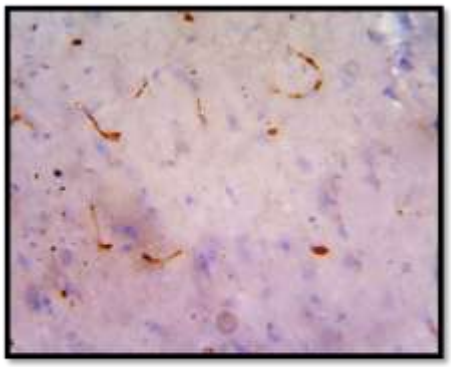


Section studied from the mouse brain [Hippocampus] of MPTP + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 163: Section of mouse brain [**Hippocampus**] from **MPTP + Captopril [Group IV]** in **MPTP model**

(40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] of MPTP + Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 164: Section of mouse brain [**Hippocampus**] from **MPTP + Perindopril** [Group V] in **MPTP model**

(40x; IHC Bcl-2)

Section studied from the mouse brain [Hippocampus] of MPTP + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 165: Section of mouse brain [**Hippocampus**] from **MPTP + Losartan [Group VI]** in **MPTP model**

(40x; IHC Bcl-2)

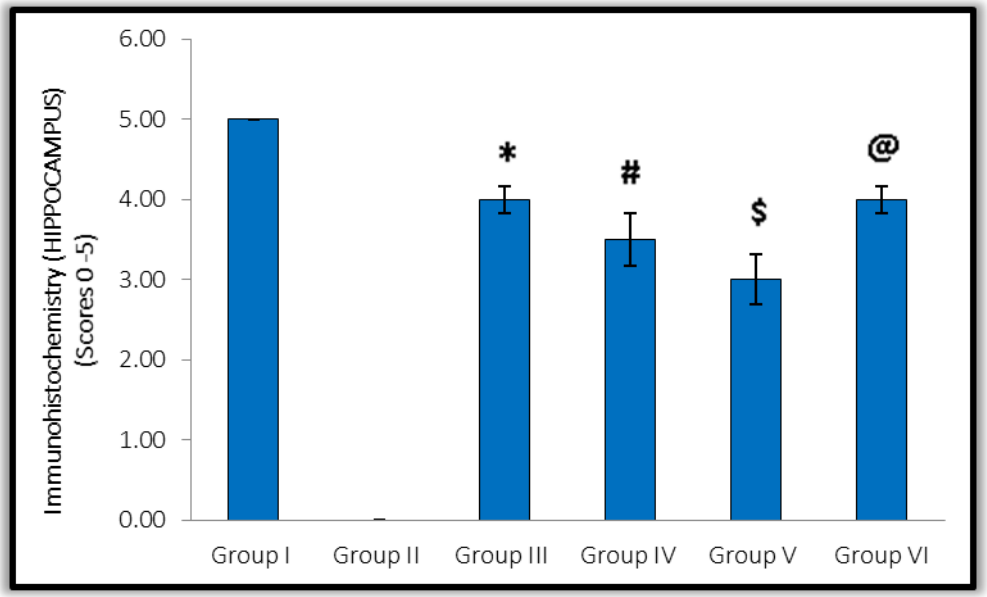
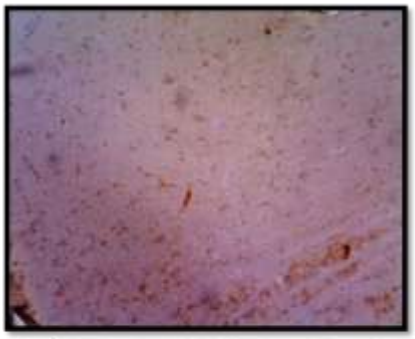


Figure 166: Bar diagram depicting the immunohistochemistry examination scores in the Hippocampus in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus were lower in the negative control group [Group II], which was statistically significant (P<0.05). It was also established that there was a statistically significant rise in the IHC scores for the hippocampus in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) as compared to the negative control group [Group II] (P<0.05).

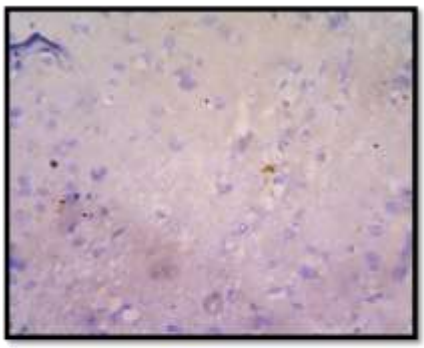
Immunohistochemistry of Prefrontal cortex [Cerebrum] in various groups of MPTP model



Section studied from the mouse brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

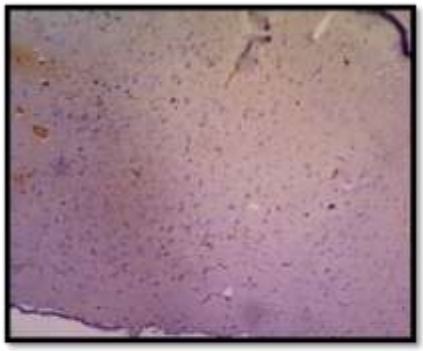
Figure 167: Section of mouse brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **MPTP model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of MPTP alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

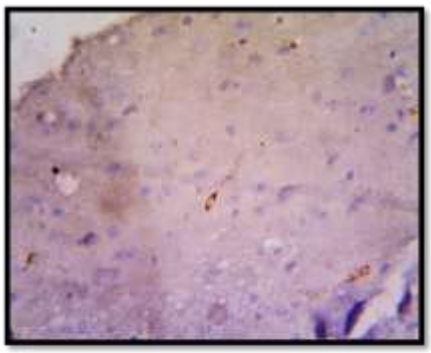
Figure 168: Section of mouse brain [**Prefrontal cortex**] from **MPTP alone group [Group II]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of MPTP + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

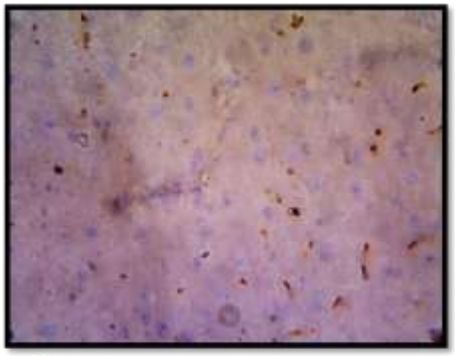
Figure 169: Section of mouse brain [**Prefrontal cortex**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of MPTP + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

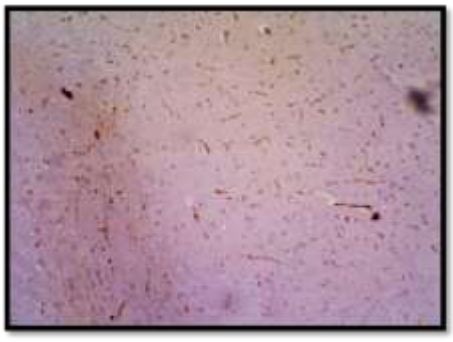
Figure 170: Section of mouse brain [**Prefrontal cortex**] from **MPTP + Captopril [Group IV]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of MPTP +Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 171: Section of mouse brain [**Prefrontal cortex**] from **MPTP +Perindopril [Group V]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of MPTP + Captoopril group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 172: Section of mouse brain [**Prefrontal cortex**] from **MPTP +Losartan [Group VI]** in **MPTP model** (10x; IHC Bcl-2)

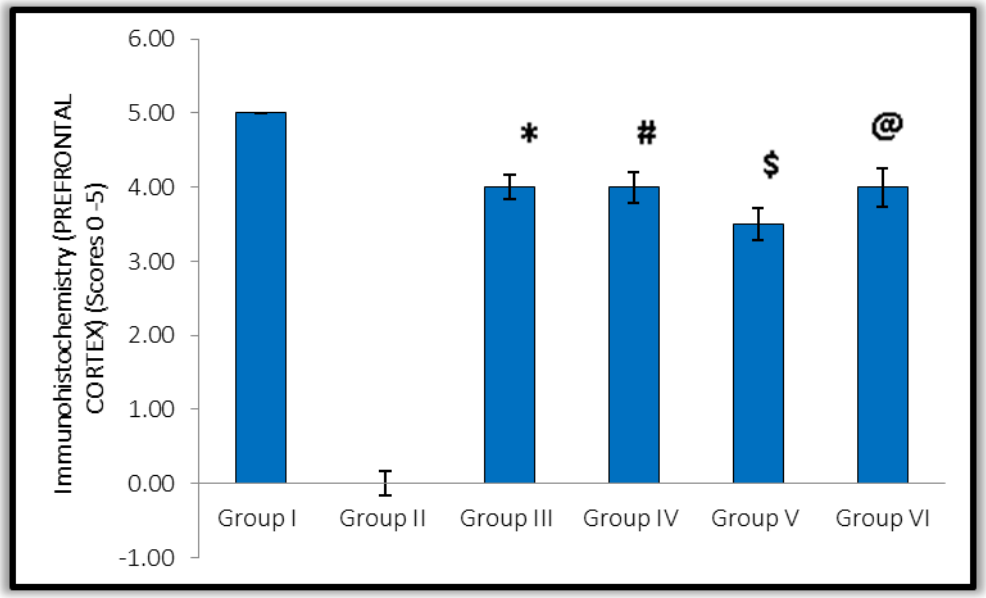
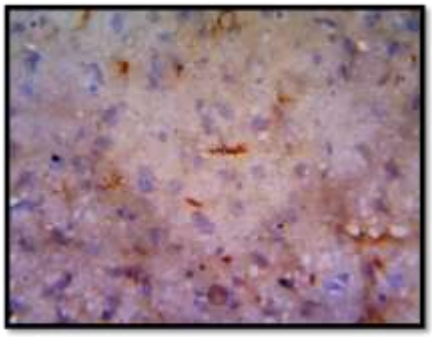


Figure 173: Bar diagram depicting the immunohistochemistry examination scores in the Prefrontal cortex in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the prefrontal cortex were lower in the negative control group [Group II], which was statistically significant (P<0.05). There was also an increase in IHC scores in the prefrontal cortex in the standard drug group [Group III] and experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

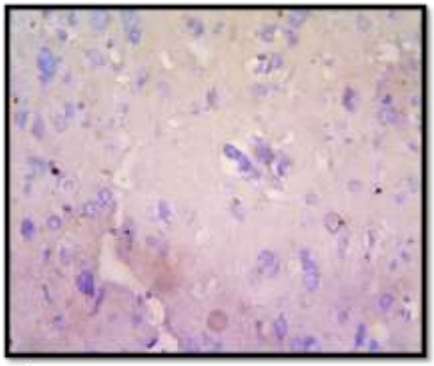
Immunohistochemistry of Corpus striatum [Basal nuclei] in various groups of MPTP model



Section studied from the mouse brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

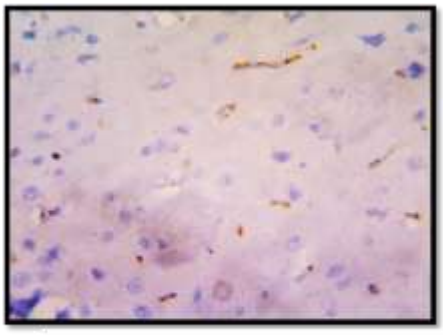
Figure 174: Section of mouse brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Pilocarpine alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

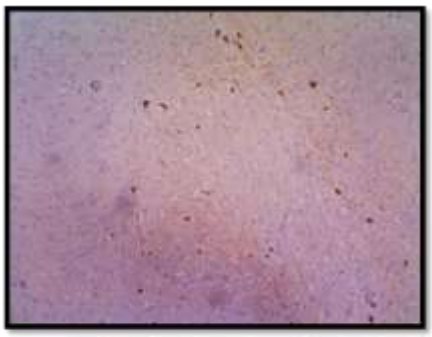
Figure 175: Section of mouse brain [**Corpus striatum**] from **MPTP alone group [Group II]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Pilocarpine + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

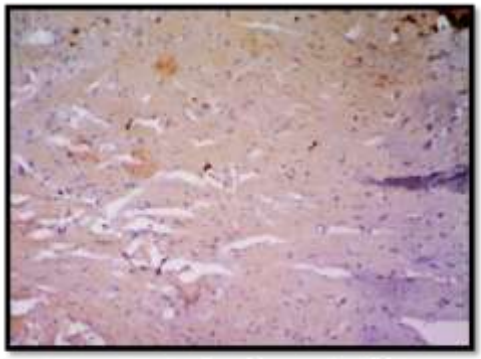
Figure 176: Section of mouse brain [**Corpus striatum**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of MPTP + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

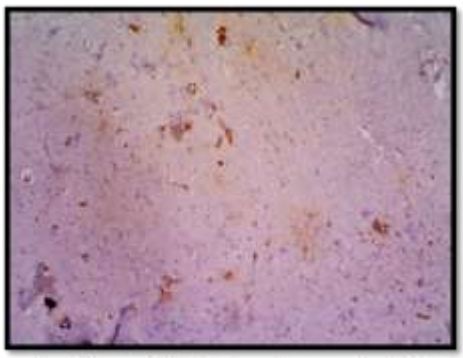
Figure 177: Section of mouse brain [**Corpus striatum**] from **MPTP + Captopril [Group IV]** in **MPTP model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of MPTP +Perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 178: Section of mouse brain [**Corpus striatum**] from **MPTP +Perindopril [Group V]** in **MPTP model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of MPTP + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 179: Section of mouse brain [**Corpus striatum**] from **MPTP + Losartan [Group VI]** in **MPTP model** (10x; IHC Bcl-2)

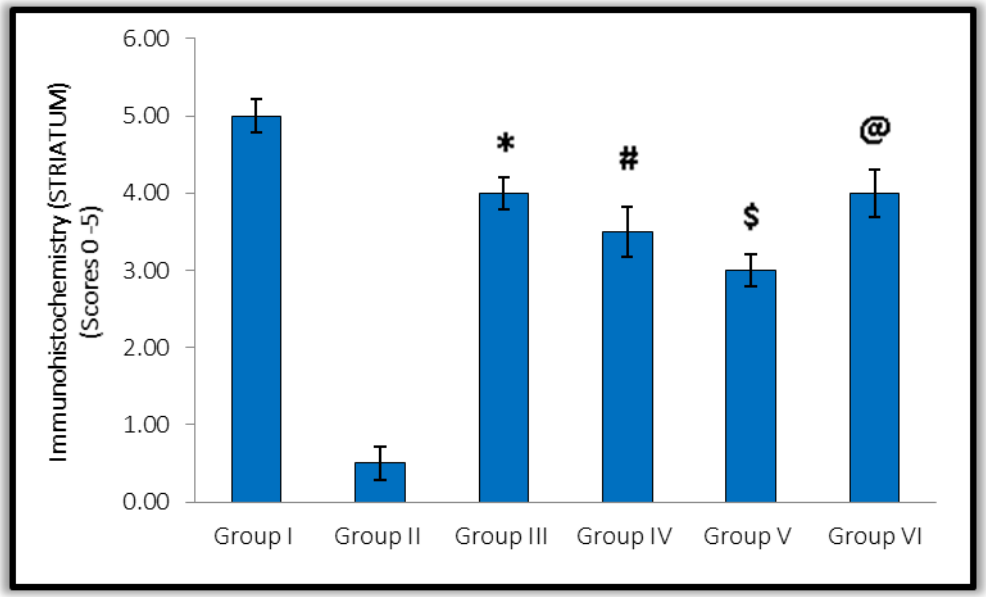
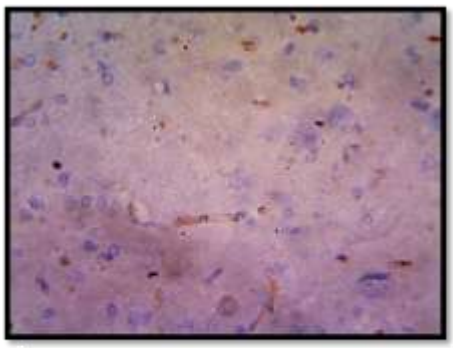


Figure 180: Bar diagram depicting the immunohistochemistry examination scores in the Corpus striatum in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the immunohistochemistry scores in the corpus striatum were lower in the negative control group [Group II], which was statistically significant (P<0.05). There was also an increase in the IHC scores in the corpus striatum in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

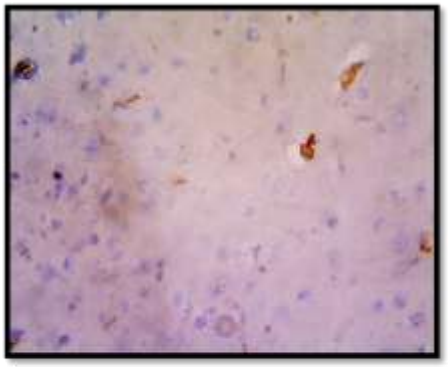
Immunohistochemistry of Hypothalamus in various groups of MPTP model



Section studied from the mouse brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

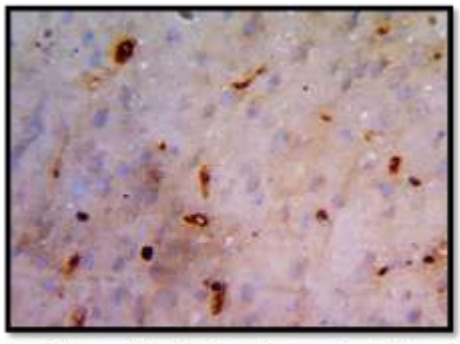
Figure 181: Section of mouse brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of MPTP alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 1.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

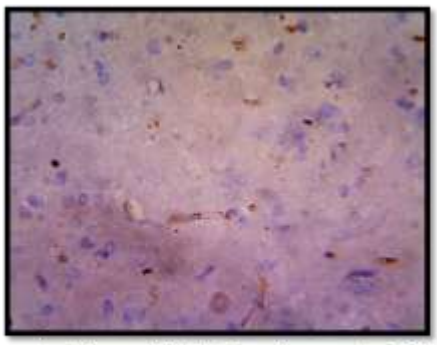
Figure 182: Section of mouse brain [**Hypothalamus**] from **MPTP alone group [Group II]** in **MPTP model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of MPTP + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.50 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 183: Section of mouse brain [**Hypothalamus**] from **MPTP + Levodopa & Benserazide [Group III]** in **MPTP model** (40x; IHC Bcl-2)

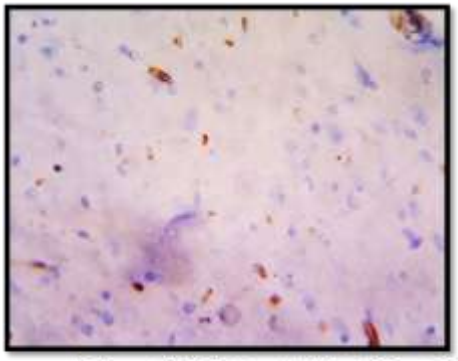


Section studied from the mouse brain [Hypothalamus] of MPTP+Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 184: Section of mouse brain [**Hypothalamus**] from **MPTP + Captopril [Group IV]** in **MPTP model**

(40x; IHC Bcl-2)

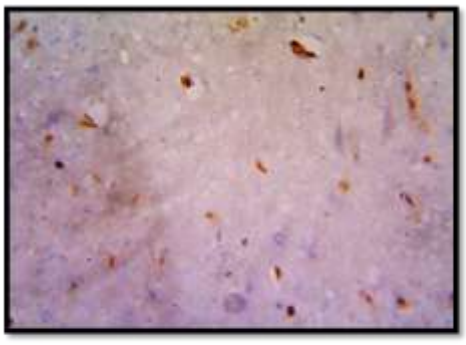


Section studied from the mouse brain [Hypothalamus] of MPTP+perindopril group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 185: Section of rat brain [**Hypothalamus**] from **MPTP + Perindopril [Group V]** in **MPTP model**

(40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of MPTP + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 186: Section of mouse brain [**Hypothalamus**] from **MPTP + Losartan [Group VI]** in **MPTP model**

(40x; IHC Bcl-2)

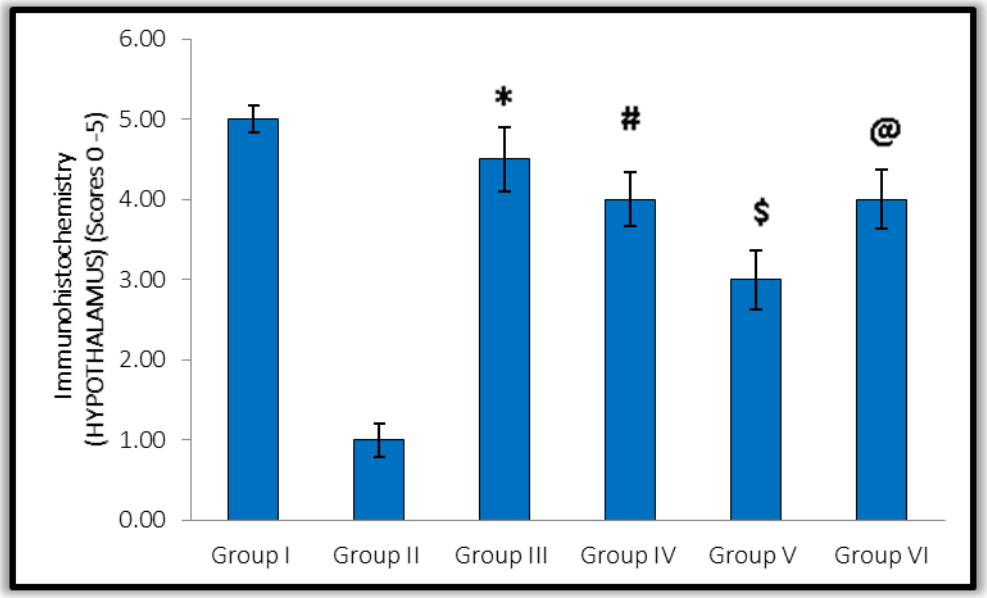


Figure 187: Bar diagram depicting the immunohistochemistry examination scores in the Hypothalamus in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the immunohistochemistry scores in the hypothalamus were lower in the negative control group [Group II], which was statistically significant (P<0.05). There was also an increase in the IHC scores in the hypothalamus in the standard drug group [Group II] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 23: Immunohistochemistry examination scores in Methyl Phenyl Tetrahydropyridine [MPTP] Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0 -5)	5.00±0.00	0.00±0.00	4.00±0.17	3.50±0.33	3.00±0.31	4.00±0.17
2	Prefrontal cortex (Scores 0 -5)	5.00±0.00	0.00±0.17	4.00±0.17	4.00±0.21	3.50±0.22	4.00±0.26
3	Corpus striatum (Scores 0 -5)	5.00±0.21	0.50±0.22	4.00±0.21	3.50±0.33	3.00±0.21	4.00±0.31
4	Hypothalamus (Scores 0 -5)	5.00±0.17	1.00±0.21	4.50±0.40	4.00±0.34	3.00±0.37	4.00±0.37

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the negative control group [Group II] to the vehicle control group [Group I], the immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were lower in the negative control group [Group II], which was statistically significant (P<0.05). There was a statistically significant increase in the IHC scores for the hippocampus, prefrontal cortex, corpus striatum and hypothalamus in the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) compared to the negative control group [Group II] (P<0.05).

III. PARAQUAT MODEL IN SWISS ALBINO MICE

Evaluation of neurobehavioral activity

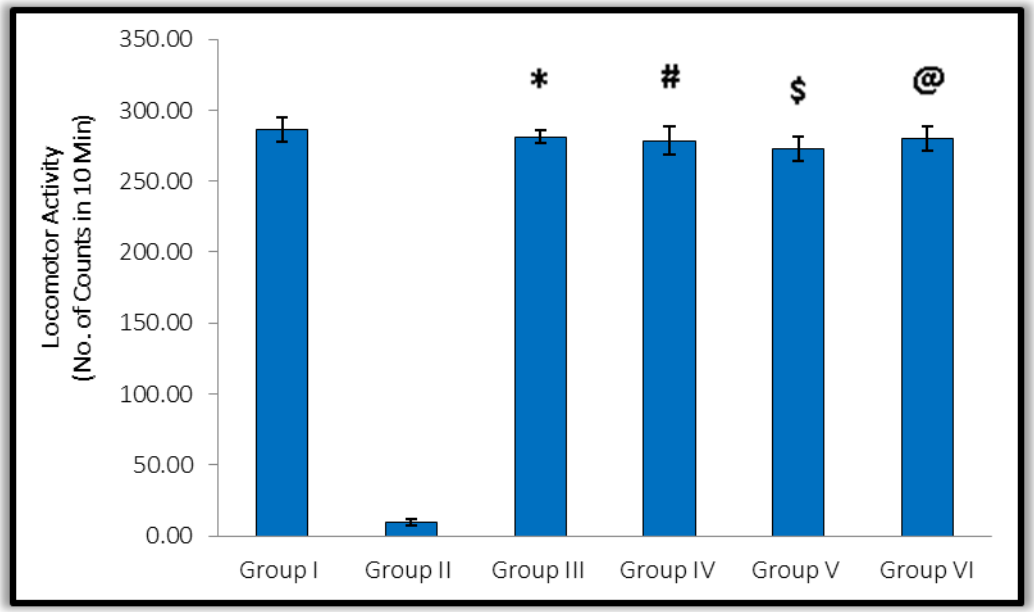


Figure 188: Bar diagram depicting the locomotor activity in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results showed that the standard group [Group III] (Levodopa+Benserazide drug) had a significant increase in the number of counts when compared to the negative control group [Group II] (P<0.05). Similarly, as compared to the negative control group [Group II], all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a rise in the total number of counts, which was statistically significant (P<0.05).

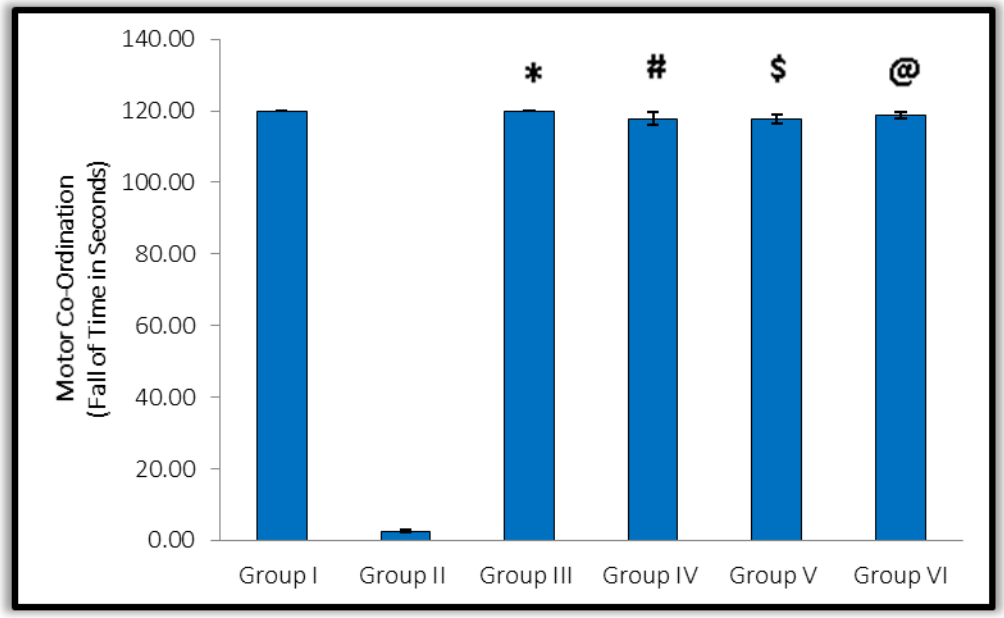


Figure 189: Bar diagram depicting the motor co-ordination in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The results showed that when comparing the standard group [Group III] (Levodopa+Benserazide drug) to the negative control group [Group II], the standard drug group [Group III] (Levodopa+Benserazide drug) had a substantial rise (P<0.05) in the fall of time. Similarly, as compared to the negative control group [Group II], all of the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increase in the fall of time, which was statistically significant (P<0.05).

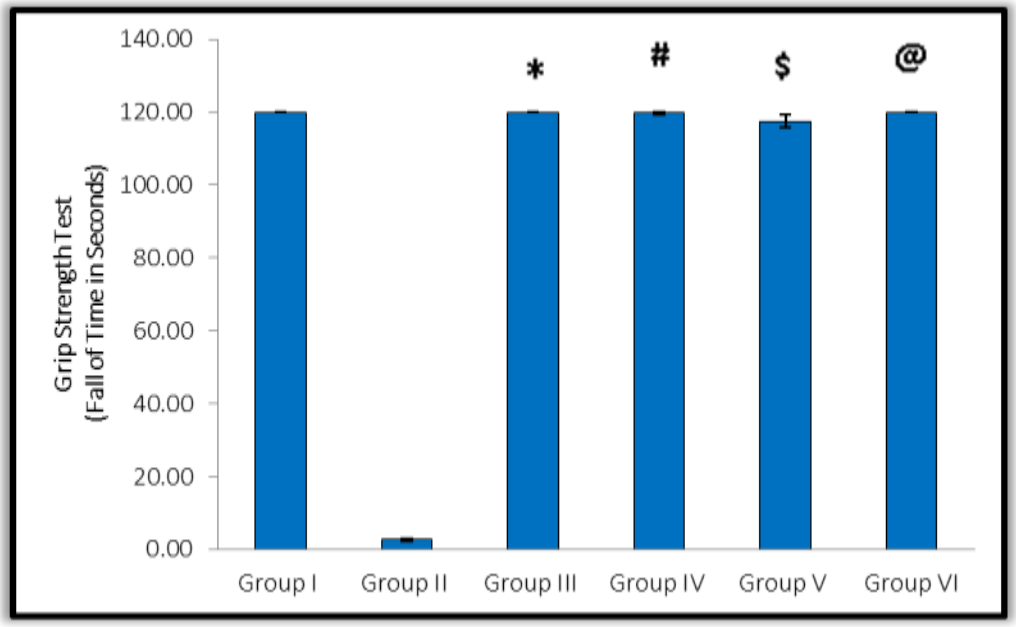


Figure 190: Bar diagram depicting the grip strength test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group [Group III] (Standard drug group) to the negative control group [Group II], there was a significant (P<0.05) rise in the parameter (fall of time in Grip Strength Test) of standard drug group [Group III]. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a substantial (P<0.05) increase in the fall of time.

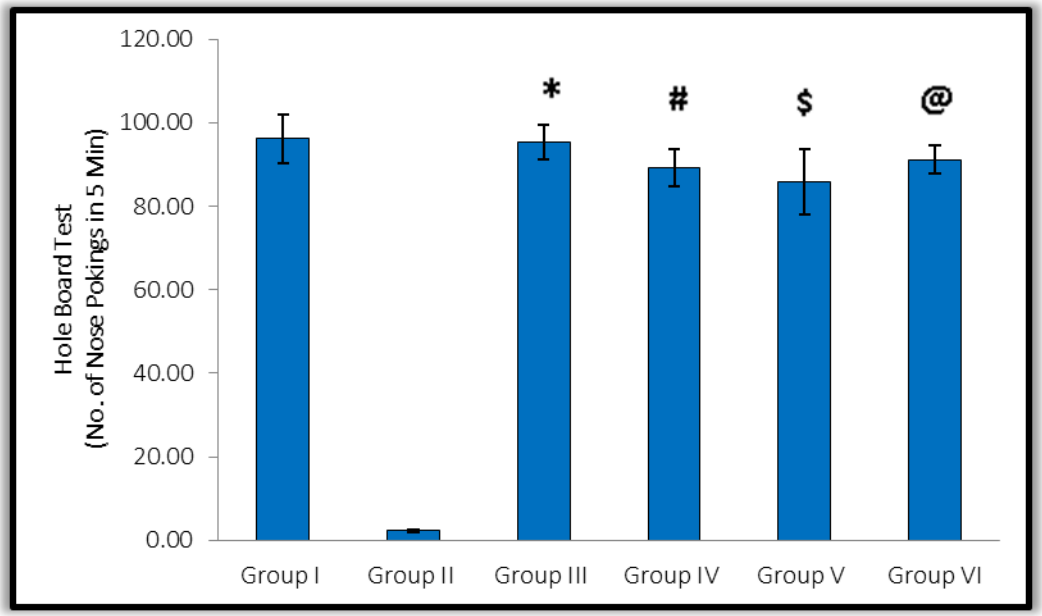


Figure 191: Bar diagram depicting the hole board test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The Levodopa+Benserazide group [Group III] (Standard drug) had a significant increase in the parameter (Nose Poking in Hole Board Test) when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) found a statistically significant (P<0.05) increase in nose poking.

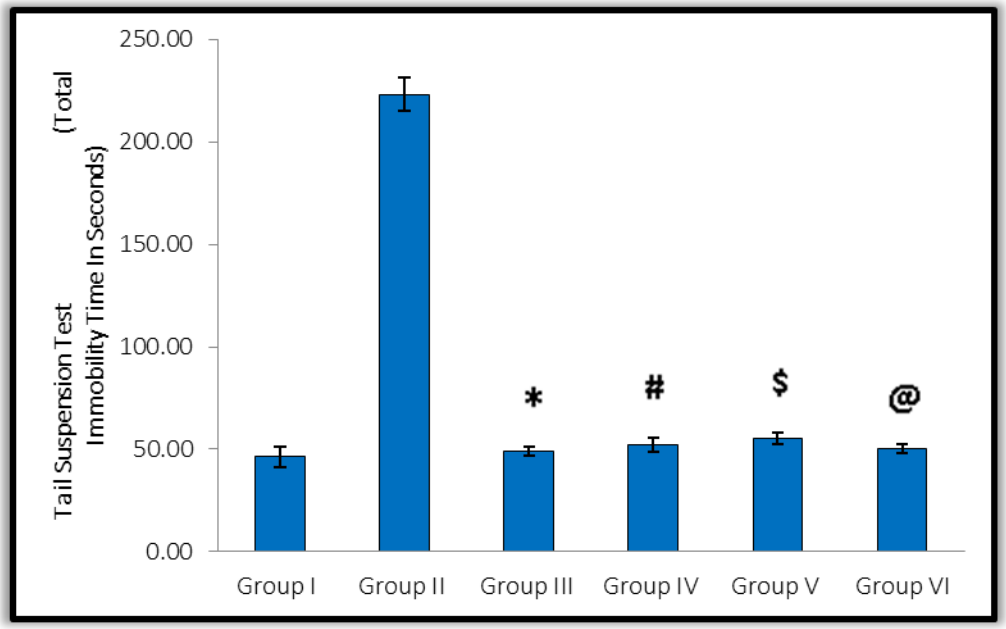


Figure 192: Bar diagram depicting the tail suspension test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant decrease in the Levodopa+Benserazide group [Group III] (Standard drug) when compared to the negative control group [Group II] (P<0.05) in the parameter (Total Immobility Time in Tail Suspension Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) proved a statistically significant (P<0.05) reduction in total immobility time.

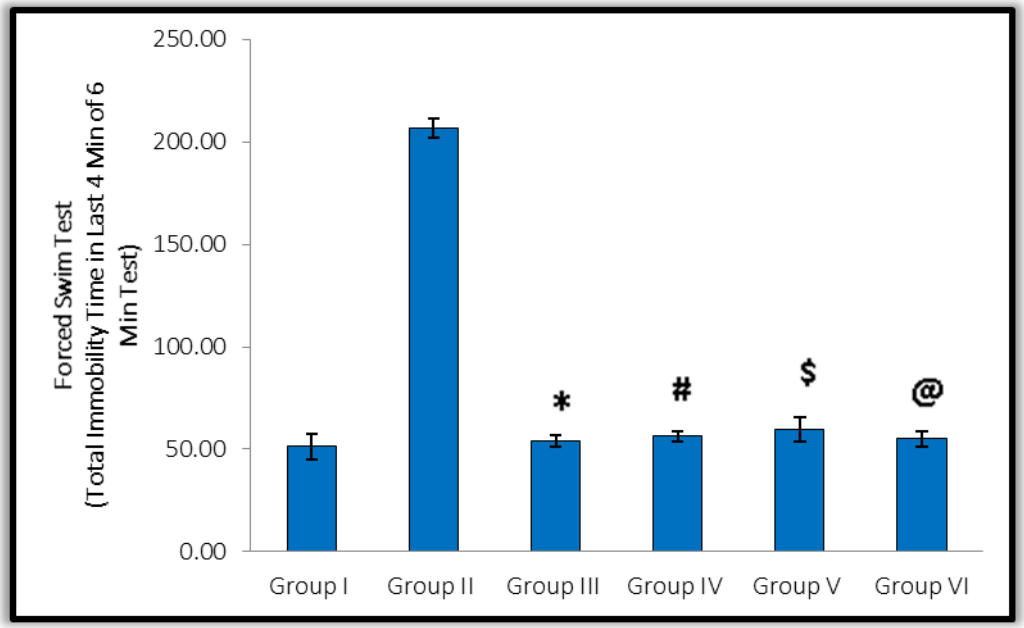


Figure 193: Bar diagram depicting the forced swim test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

In the parameter (Total Immobility Time in Forced Swim Test), the Levodopa+Benserazide group [Group III] (Standard drug) showed a significant decrease when compared to the negative control group [Group II] (P<0.05). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant (P<0.05) reduction in total immobility time.

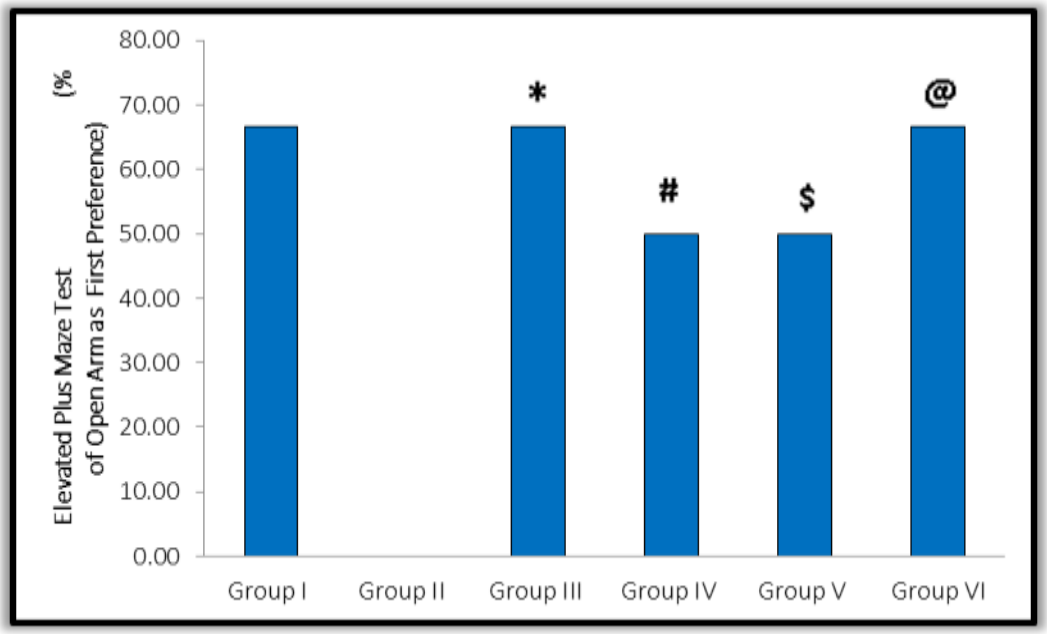


Figure 194: Bar diagram depicting the percentage of open arm as first preference in elevated plus maze test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group [Group III] (Standard drug) to the negative control group [Group II], there was a substantial (P<0.05) increase in the percentage of open arms as the first arm preference in the Elevated Plus Maze Test. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) evidenced a statistically significant (P<0.05) increase in the percentage of open arms as first arm preference.

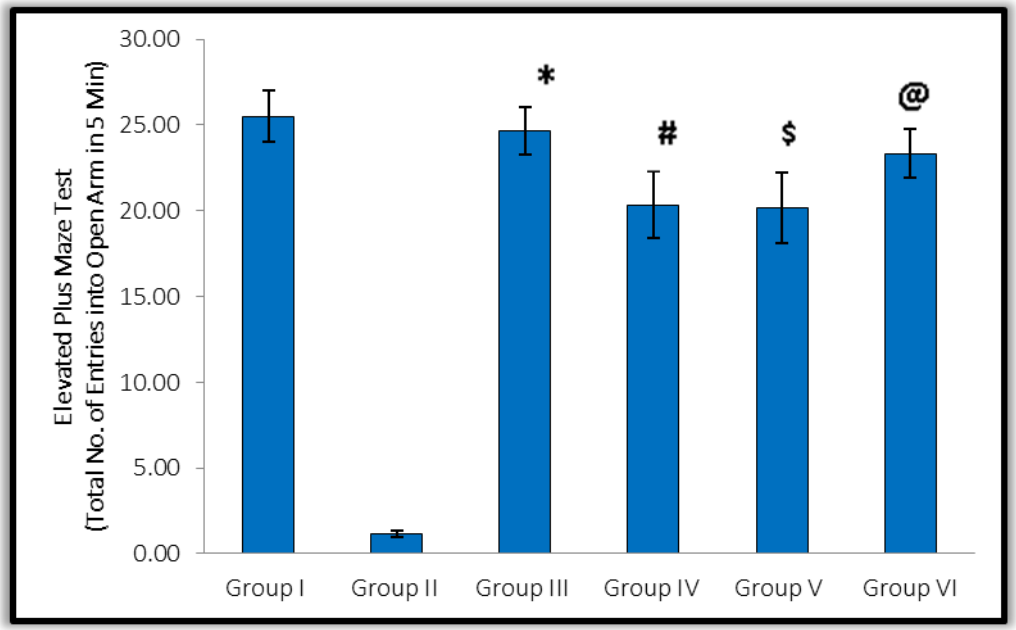


Figure 195: Bar diagram depicting the total number of entries into the open arm in elevated plus maze test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant increase in the Levodopa+Benserazide group [Group III] (Standard drug) as compared to the negative control group [Group II] (P<0.05) in the parameter (Total Number of Entries into the Open Arm in the Elevated Plus Maze Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) indicated a statistically significant (P<0.05) increase in the total number of entries into the open arm.

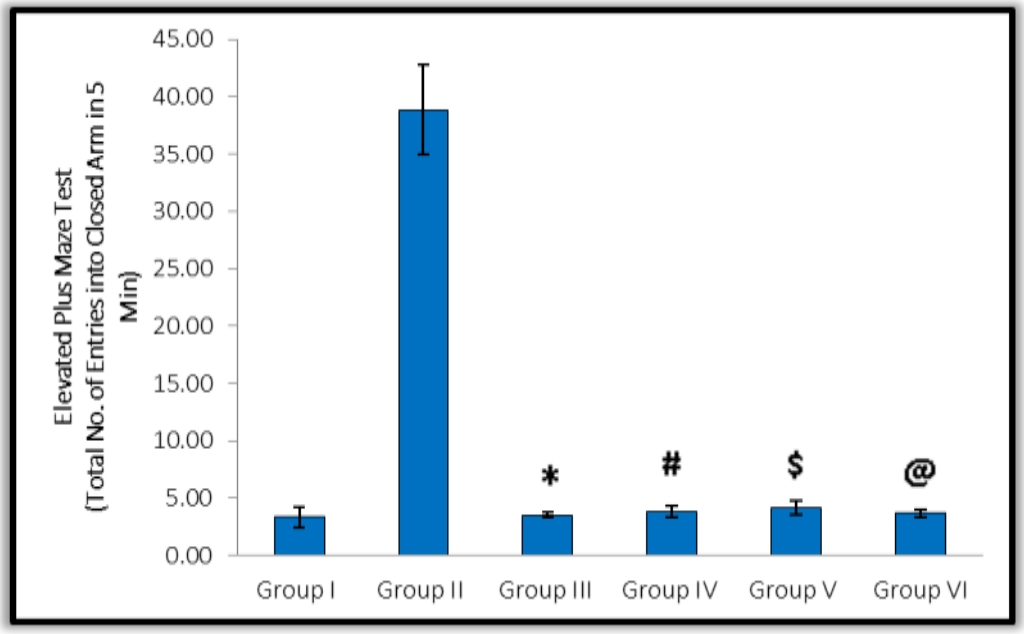


Figure 196: Bar diagram depicting the total number of entries into the closed arm in elevated plus maze test in Paraquat model screening test in Swiss Albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

There was a significant decrease in the Levodopa+Benserazide group [Group III] (Standard drug) when compared to the negative control group [Group II] (P<0.05) in the parameter (Total Number of Entries into Closed Arm in Elevated Plus Maze Test). When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant (P<0.05) drop in the total number of entries into the closed arm.

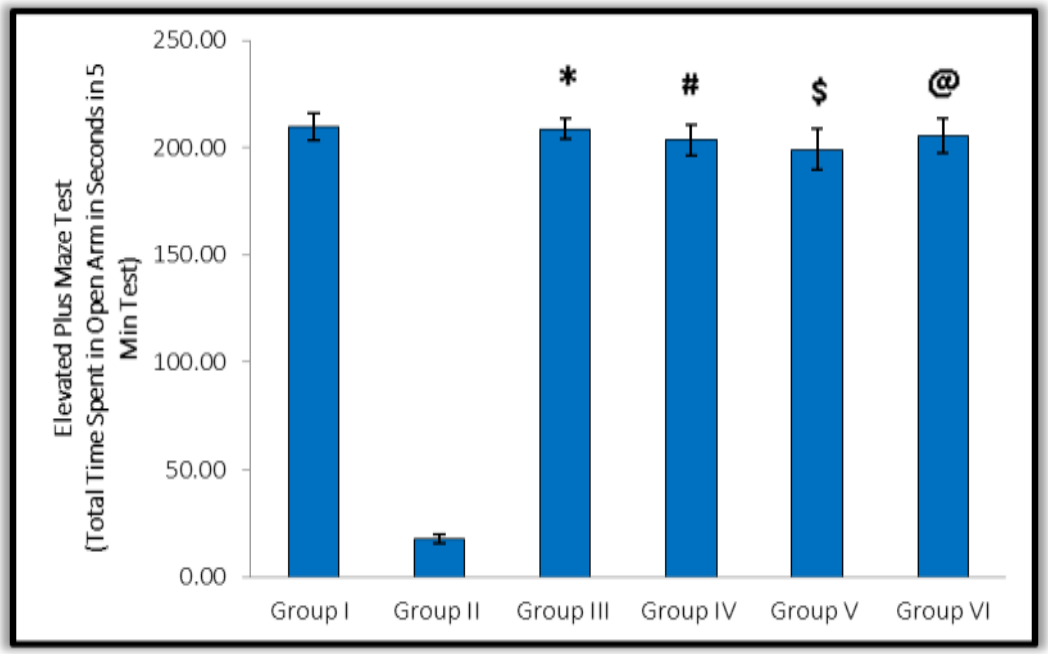


Figure 197: Bar diagram depicting the total time spent in the open arm in elevated plus maze test in Paraquat model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When comparing the Levodopa+Benserazide group [Group III] (Standard drug) to the negative control group [Group II], the parameter (Time Spent In Open Arm in the Elevated Plus Maze Test) showed a significant (P<0.05) increase in the standard drug group [Group III]. When compared to the negative control group [Group II], the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistical significant (P<0.05) increase in the time spent in open arm when compared to the negative control group [Group II].

Table 24: Behavioural analysis in Paraquat model

Group	Actophotometer test	Rotarod test	Grip strength test	Hole board test	Tail suspension test	Force swim test	Elevated plus maze			
							% Open arm preference	No. of entries into the open arm	No. of entries into the close arm	Time spent in the open arm
I [Vehicle control]	286.50±8.77	120.00±0.00	120.00±0.00	96.17±5.88	46.50±4.97	51.33±6.08	66.67	25.50±1.48	3.33±0.92	209.50±6.37
II [Negative control]	9.67±1.93	2.50±0.34	2.67±0.33	2.33±0.33	223.17±8.25	206.50±4.61	0.00	1.17±0.17	38.83±3.90	17.67±1.94
III [Standard control]	281.17±4.69	120.00±0.00	120.00±0.00	95.33±4.23	49.17±2.10	53.83±2.89	66.67	24.67±1.41	3.50±0.22	208.67±4.93
IV [Captopril]	278.33±9.94	117.83±1.64	119.67±0.33	89.17±4.48	52.17±3.68	56.33±2.67	50.00	20.33±1.94	3.83±0.48	203.50±7.39
V [Perindopril]	272.83±8.82	117.67±1.38	117.50±1.71	85.83±7.75	55.17±2.69	59.83±6.02	50.00	20.17±2.06	4.17±0.60	199.17±9.49
VI [Losartan]	280.33±8.69	118.67±0.84	120.00±0.00	91.17±3.41	50.33±2.42	55.17±3.75	66.67	23.33±1.41	3.67±0.33	205.33±8.13

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Evaluation of oxidative stress markers

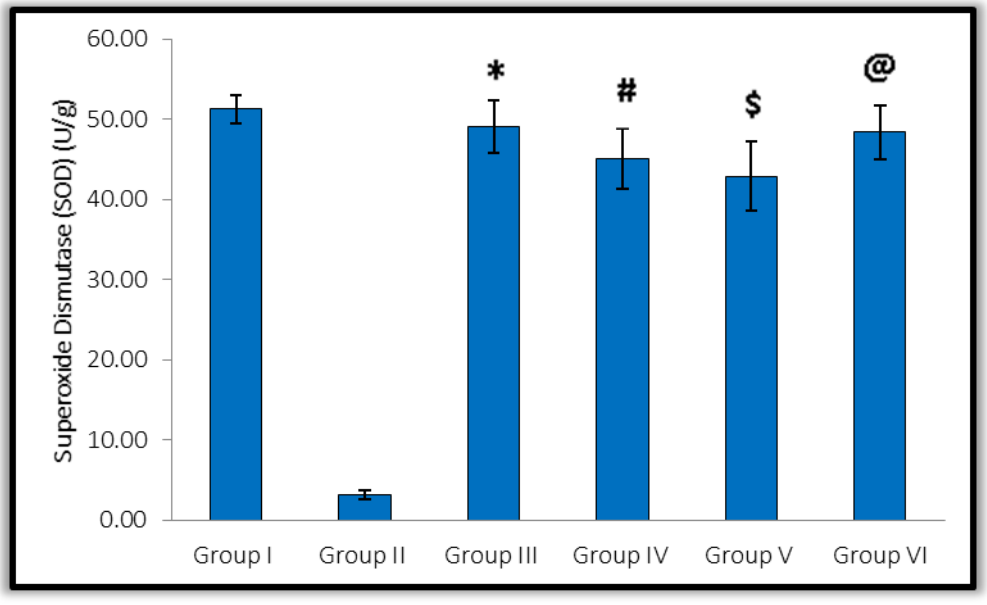


Figure 198: Bar diagram depicting the anti-oxidant enzyme (*Superoxide dismutase*) level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Superoxide dismutase) level in the negative control group [Group II] was decreased significantly (P<0.05). The study found that the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed an increase in the anti-oxidant enzyme (*Superoxide dismutase*) level and it was found to be statistically significant when compared to the negative control group [Group II] (P<0.05).

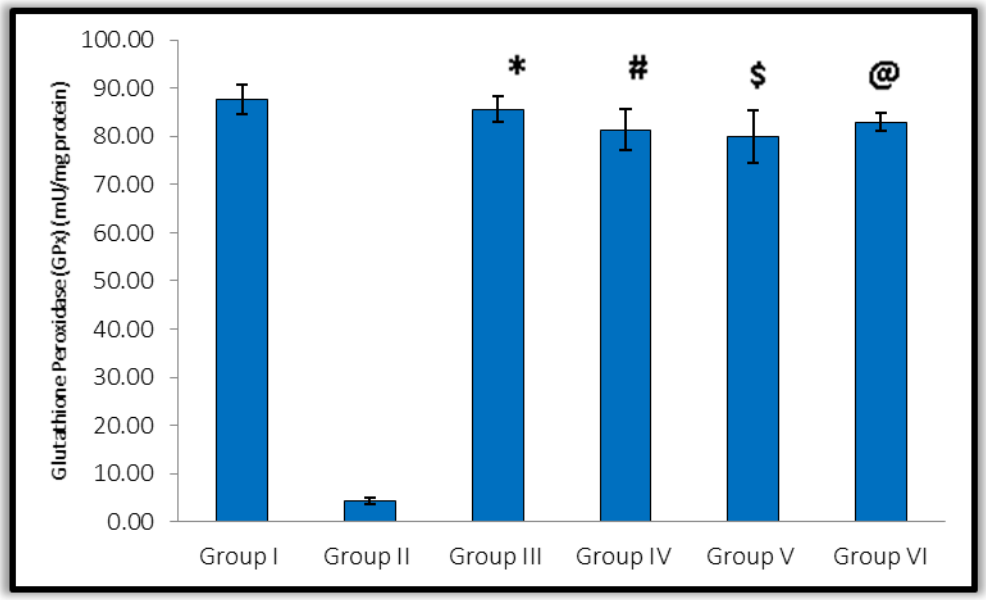


Figure 199: Bar diagram depicting the anti-oxidant enzyme (*Glutathione peroxidase*) level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Similarly, when compared to the vehicle control group [Group I], the anti-oxidant enzyme (glutathione peroxidase) level was decreased in the negative control

group [Group II] (P<0.05). Additionally, the study results indicated that the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased the level of an anti-oxidant enzyme (Glutathione peroxidase), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

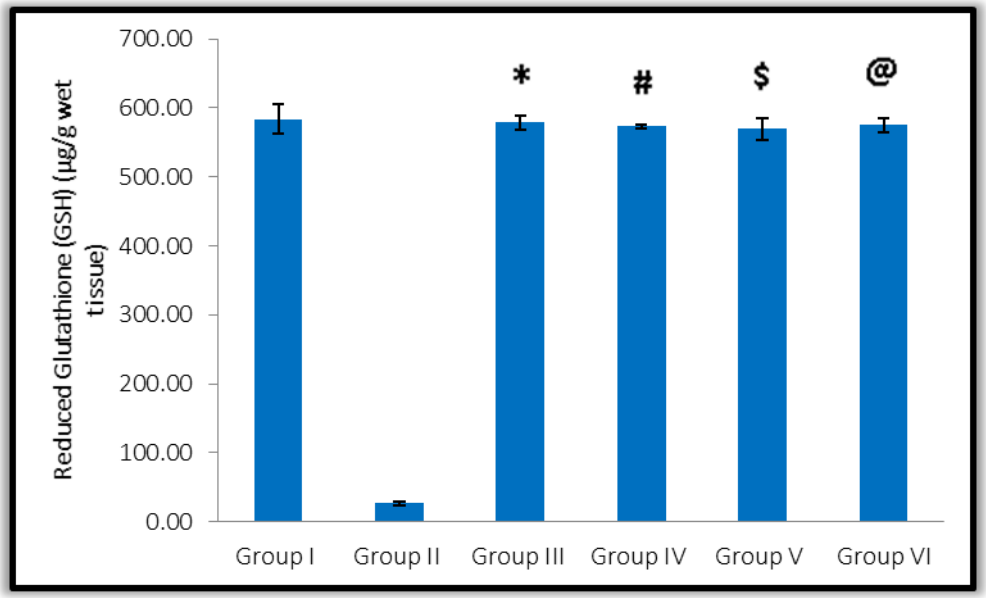


Figure 200: Bar diagram depicting the anti-oxidant enzyme (*Reduced glutathione*) level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (reduced glutathione) level was decreased in the negative control group [Group II] (P<0.05). Additionally, the analysis found that both the standard drug [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased the level of an anti-oxidant enzyme

(reduced glutathione), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

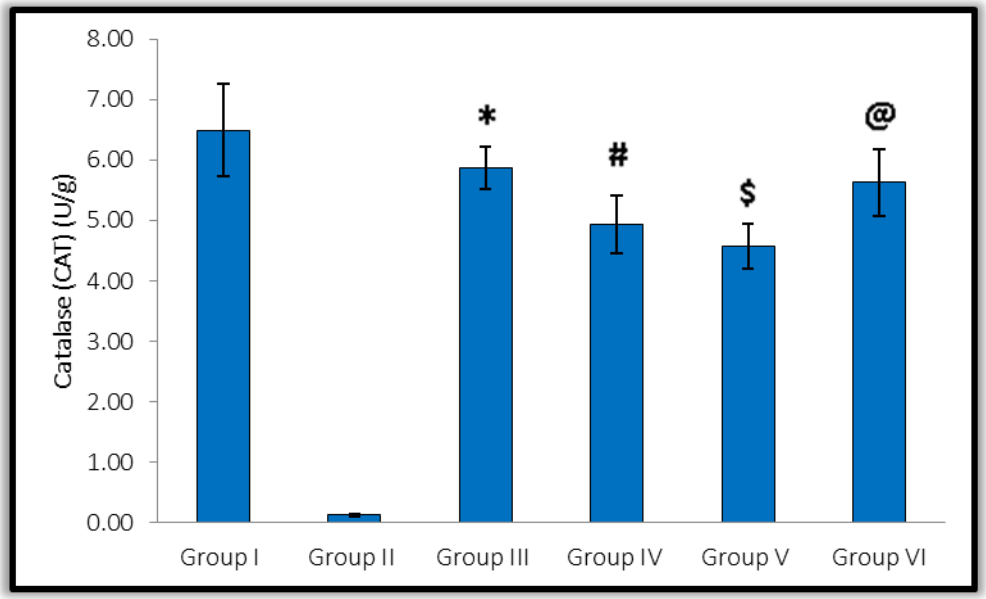


Figure 201: Bar diagram depicting the anti-oxidant enzyme (*Catalase*) level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], the anti-oxidant enzyme (Catalase) level was decreased in the negative control group [Group II] (P<0.05). Also, the study observed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) significantly increased the anti-oxidant enzyme (Catalase) level when compared to the negative control group [Group II] (P<0.05).

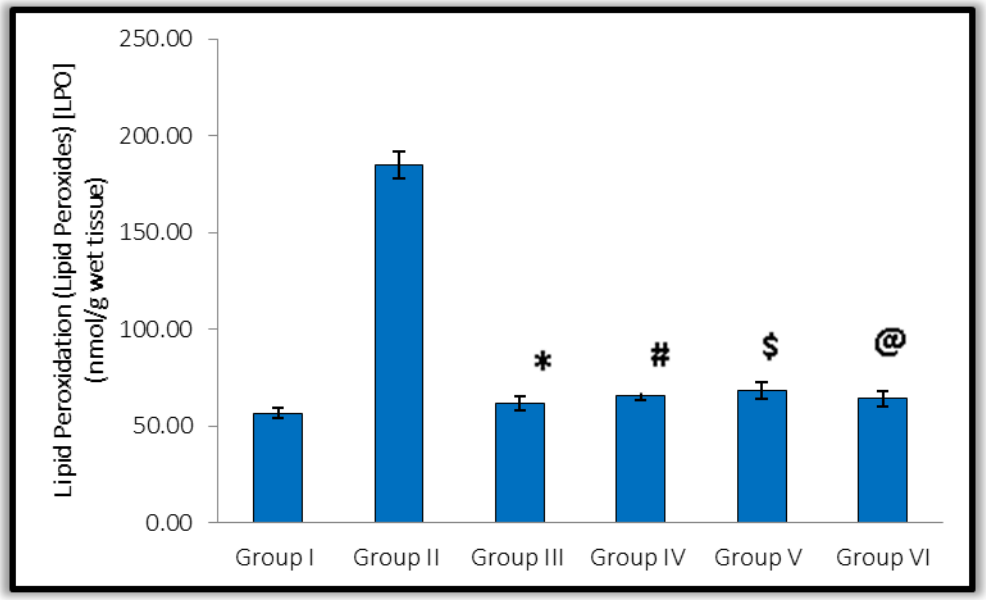


Figure 202: Bar diagram depicting the *lipid peroxidation* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

When compared to the vehicle control group [Group I], lipid peroxidation was remarkably higher in the negative control group [Group II] (P<0.05). Further, the study determined that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) significantly reduced lipid peroxidation when compared to the negative control group [Group II] (P<0.05).

Table 25: Anti-oxidant enzymes in Paraquat Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Superoxide Dismutase (SOD) (*U/g*)	51.23±1.72	3.20±0.61	49.10±3.32	45.07±3.78	42.88±4.28	48.37±3.41
2	Glutathione Peroxidase (GPx) (*mU/mg protein*)	87.62±3.09	4.29±0.65	85.58±2.68	81.34±4.28	79.97±5.37	82.97±1.94
3	Reduced Glutathione (GSH) (*µg/g wet tissue*)	583.65±21.29	26.59±2.96	578.54±10.76	573.28±2.61	569.80±15.66	575.22±10.65
4	Catalase (CAT) (*U/g*)	6.49±0.76	0.12±0.02	5.87±0.35	4.93±0.47	4.57±0.37	5.63±0.55
5	Lipid Peroxidation (LPO) (*nmol/g wet tissue*)	56.65±2.54	184.81±6.81	61.43±3.65	65.52±2.31	68.36±4.52	64.28±4.01

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The levels of all anti-oxidant enzymes (Superoxide Dismutase, Glutathione Peroxidase, Reduced Glutathione and Catalase) were significantly lower in the negative control group [Group II] than in the vehicle control group [Group I] (P<0.05). Correspondingly, the study uncovered that both the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased anti-oxidant enzyme levels (Superoxide Dismutase, Glutathione Peroxidase, Reduced Glutathione and Catalase), which was statistically significant when compared to the negative control group [Group II] (P<0.05).

But the level of MPO was significantly increased in the negative control group [Group II] than in the vehicle control group [Group I] (P<0.05). Correspondingly, the study found that both the standard drug group [Group III] and the experimental

groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) decreased the MPO level which was statistically significant when compared to the negative control group [Group II] ($P<0.05$).

Evaluation of neurotransmitters

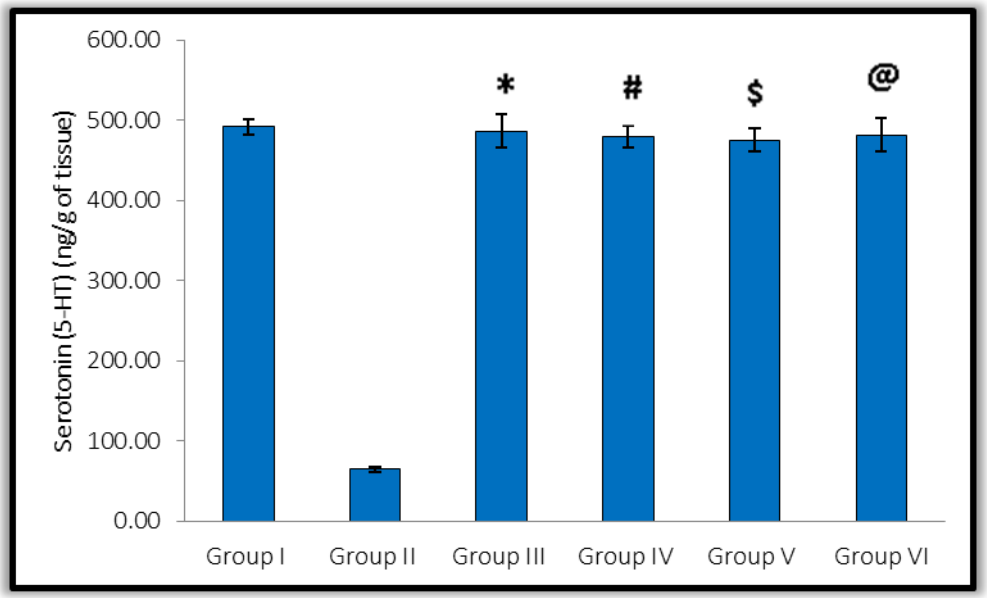


Figure 203: Bar diagram depicting the *Serotonin* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (Serotonin) level was significantly ($P<0.05$) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Likewise, it was observed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased the neurotransmitter (Serotonin) level statistically significantly ($P<0.05$) when compared to the negative control group [Group II].

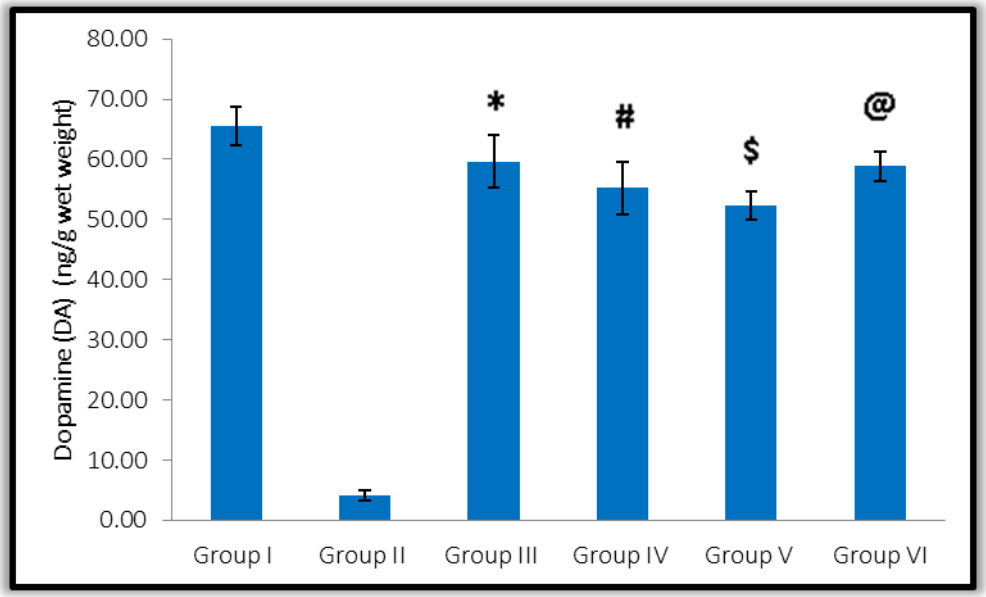


Figure 204: Bar diagram depicting the *Dopamine* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (Dopamine) level was significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. Further, it was observed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased the neurotransmitter (Dopamine) level substantially (P<0.05) when compared to the negative control group [Group II].

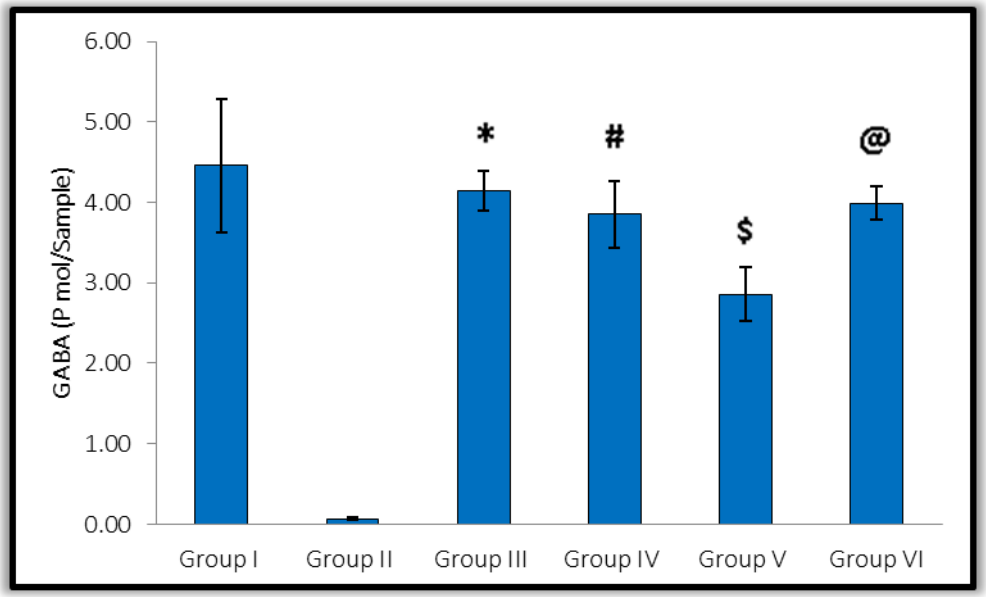


Figure 205: Bar diagram depicting the *GABA* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (GABA) level was significantly lower in the negative control group [Group II] than in the vehicle control group [Group I]. Meanwhile, the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistically significant rise in neurotransmitter (GABA) levels as compared to the negative control group [Group II] ($P<0.05$).

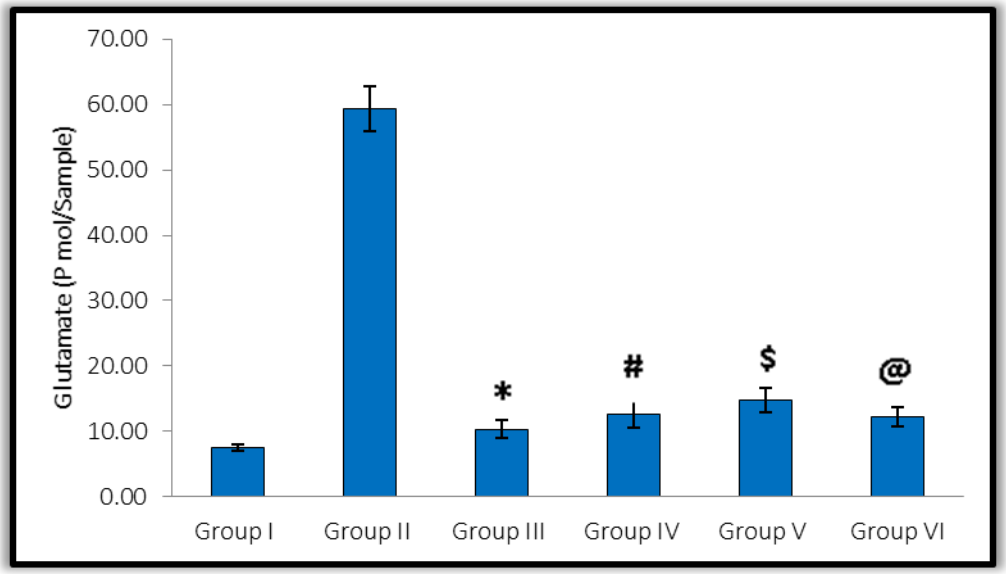


Figure 206: Bar diagram depicting the *Glutamate* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (glutamate) level was significantly elevated in the negative control group [Group II] when compared to the vehicle control group [Group I]. Additionally, it was observed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) all proved a statistically significant decrease in the neurotransmitter (Glutamate) level when compared to the negative control group [Group II] (P<0.05).

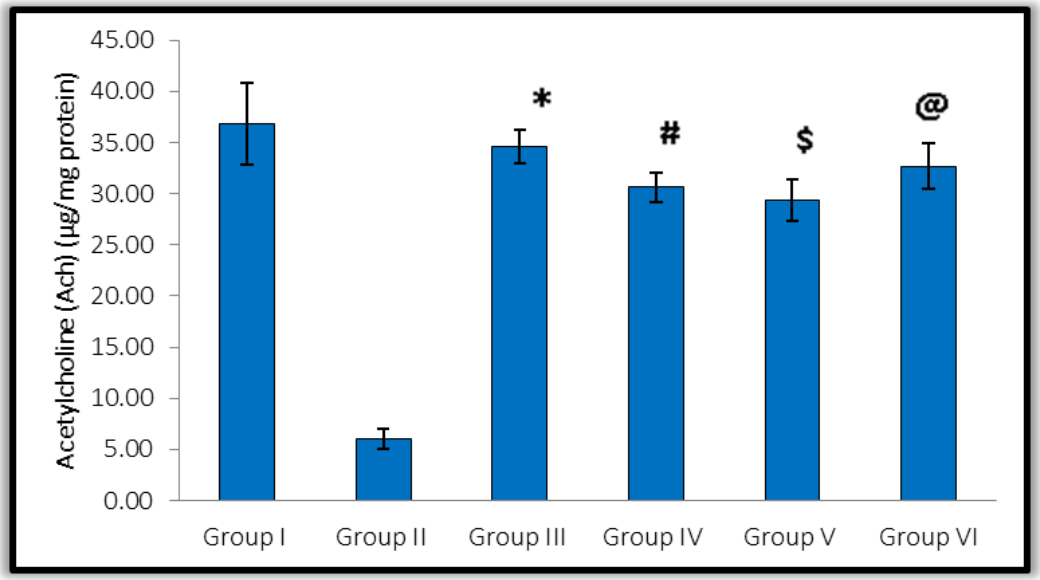


Figure 207: Bar diagram depicting the *Acetylcholine* level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The neurotransmitter (ACh) level was significantly decreased in the negative control group [Group II] compared to the vehicle control group [Group I]. Additionally, the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) presented a statistically significant rise in the neurotransmitter (ACh) level when compared to the negative control group [Group II] (P<0.05).

Table 26: Neurotransmitters in Paraquat Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Serotonin (*ng/g of tissue*)	491.84±9.82	64.83±3.34	486.52±21.26	479.77±13.35	474.83±14.29	481.18±20.87
2	Dopamine (*ng/g wet weight*)	65.50±3.23	4.06±0.84	59.64±4.30	55.27±4.39	52.38±2.37	58.84±2.47
3	GABA (*P mol/Sample*)	4.46±0.83	0.07±0.02	4.14±0.25	3.85±0.41	2.86±0.34	3.99±0.21
4	Glutamate (*P mol/Sample*)	7.53±0.45	59.35±3.41	10.37±1.40	12.62±2.07	14.85±1.87	12.24±1.38
5	Acetylcholine (*µg/mg protein*)	36.85±4.03	6.01±0.95	34.57±1.61	30.66±1.44	29.37±2.02	32.68±2.22

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The levels of neurotransmitters (5-HT, DA, GABA and ACh) were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Likewise, it was observed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased neurotransmitter (5-HT, DA, GABA and ACh) levels in a statistically significant manner when compared to the negative control group [Group II] (P<0.05).

The neurotransmitter (glutamate) level was significantly (P<0.05) elevated in the negative control group [Group II] when compared to the vehicle control group [Group I]. Furthermore, it was observed that the standard drug group [Group III] and experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) revealed a statistically significant decrease in neurotransmitter (Glutamate) levels when compared to the negative control group [Group II] (P<0.05).

Evaluation of inflammatory marker

Myeloperoxidase [MPO]

Table 27: Evaluation of inflammatory marker [MPO] in Paraquat model

Group	I [Vehicle control]	II [Negative control]	III [Standard control]	IV [Captopril]	V [Perindopril]	VI [Losartan]
MPO	0.53±0.11	10.67±0.95	1.45±0.29	1.81±0.18	2.17±0.16	1.64±0.39

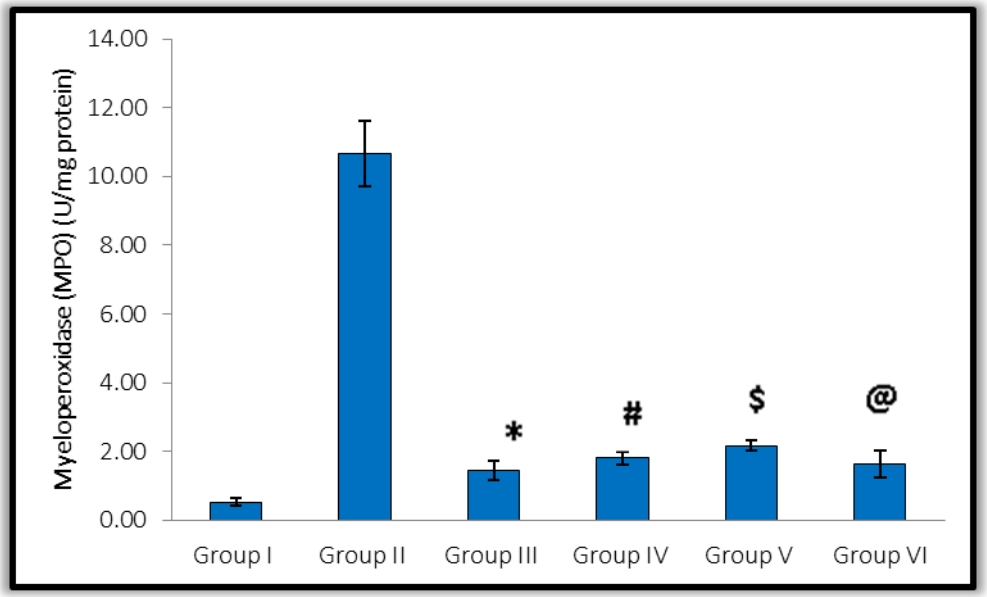


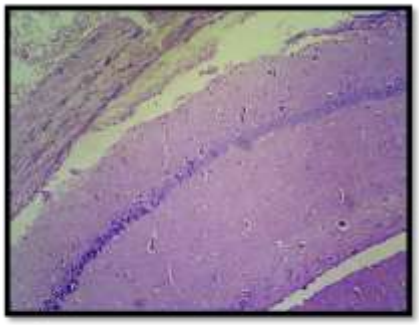
Figure 208: Bar diagram depicting the inflammatory marker (*Myeloperoxidase*) level in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a significantly higher level of myeloperoxidase than the vehicle control group [Group I], which was statistically significant (P<0.05). Moreover, it was demonstrated that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) all exhibited a statistically significant decrease in Myeloperoxidase levels when compared to the negative control group [Group II] (P<0.05).

MICROANATOMICAL [HISTOPATHOLOGICAL] STUDY IN PARAQUAT MODEL

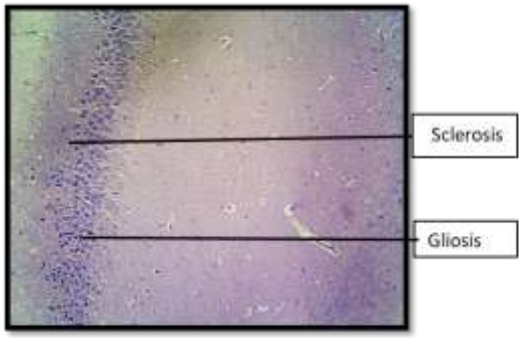
Microanatomical [Histopathological] study of Hippocampus in various groups of Paraquat model



Section studied from the mouse brain [Hippocampus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

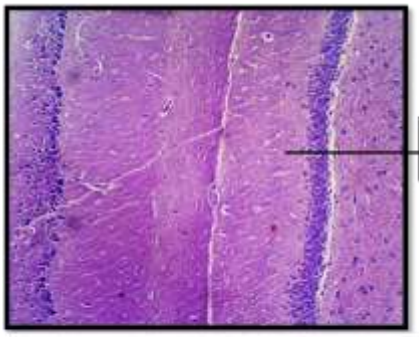
Figure 209: Section of mouse brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Paraquat model** (10x; H &E stained)



Section studied from the mouse brain [Hippocampus] of Paraquat alone group [Group II] showing severe gliosis and sclerosis and gliosis amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

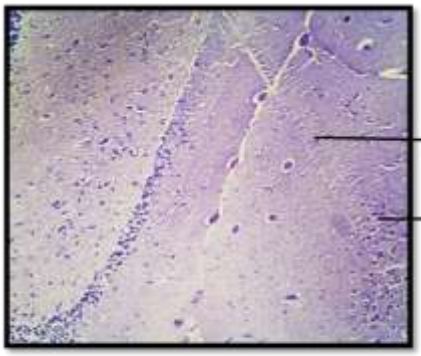
Figure 210: Section of mouse brain [**Hippocampus**] from **Paraquat alone group [Group II]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Hippocampus] of Paraquat + Positive control group [Group III] showing mild mild degeneration and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 211: Section of mouse brain [**Hippocampus**] from **Paraquat+ Levodopa & Benserazide [Group III]** in **Paraquat model** (10x; H & E stained)

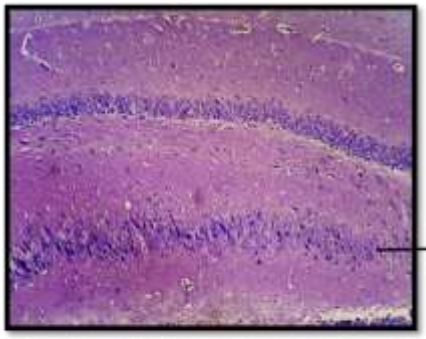


Section studied from the mouse brain [Hippocampus] of Paraquat + Captopril group [Group IV] showing moderate gliosis and sclerosis and sclerosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 212 : Section of mouse brain [**Hippocampus**] from **Paraquat+ Captopril [Group IV]** in **Paraquat model**

(10x; H & E stained)

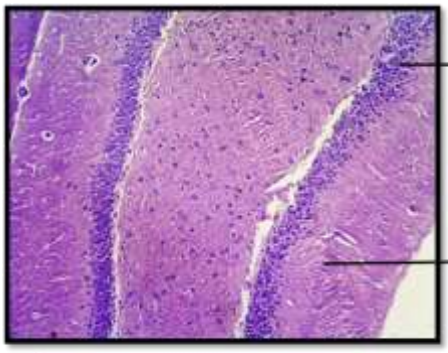


Section studied from the mouse brain [Hippocampus] of Paraquat + Perindopril group [Group V] showing mild gliosis amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 213: Section of mouse brain [**Hippocampus**] from **Paraquat+ Perindopril [Group V]** in **Paraquat model**

(10x; H & E stained)



Section studied from the mouse brain [Hippocampus] of Paraquat + Losartan group [Group VI] showing mild gliosis and degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 214: Section of mouse brain [**Hippocampus**] from **Paraquat + Losartan [Group VI]** in **Paraquat model**

(10x; H & E stained

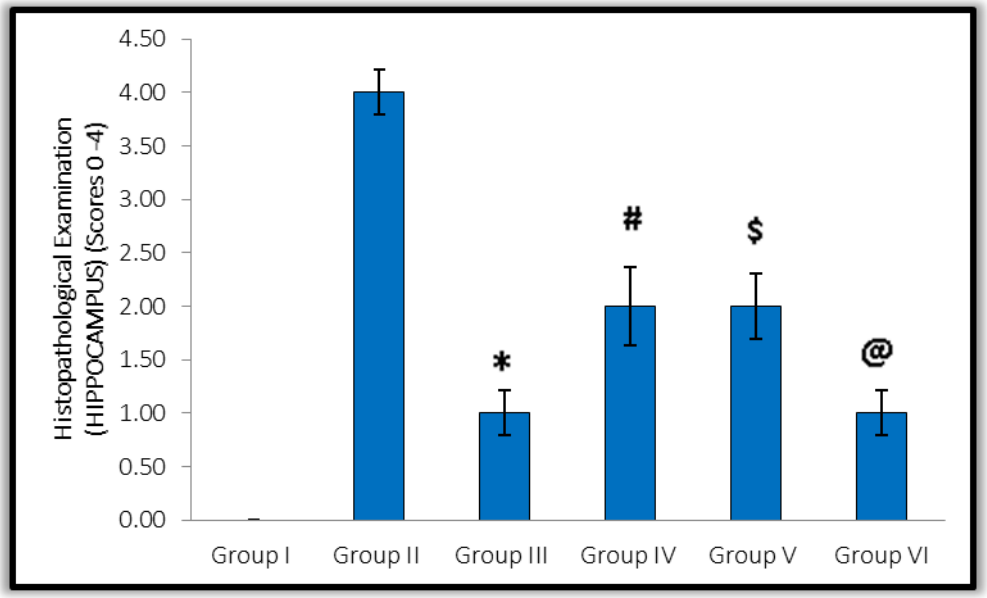
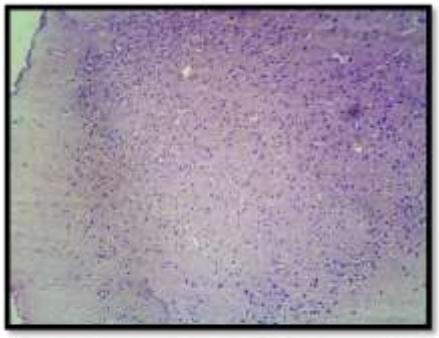


Figure 215: Bar diagram depicting the histopathological examination scores in Hippocampus in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a statistically significant (P<0.05) increase in histopathological scores in the hippocampus when compared to the vehicle control group [Group I]. Additionally, it was noticed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistically significant decrease in histopathological scores in the hippocampus (P<0.05) when compared to the negative control group [Group II].

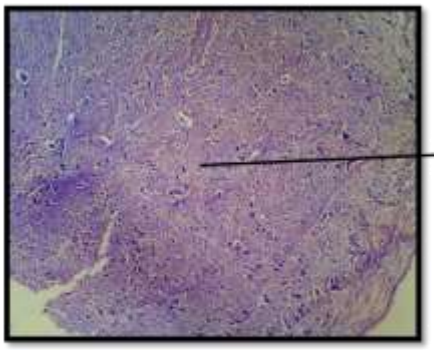
Microanatomical [Histopathological] examination of Prefrontal cortex [Cerebrum] in Paraquat model



Section studied from the rat brain [Prefrontal cortex] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

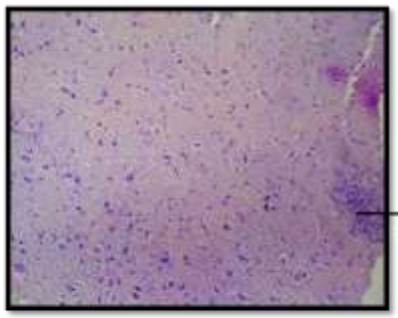
Figure 216: Section of rat brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Paraquat model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Paraquat alone group [Group II] showing sclerosis amounting to the histopathological score 4.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

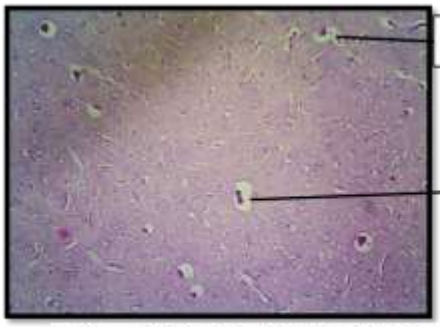
Figure 217: Section of rat brain [**Prefrontal cortex]** from **Paraquat alone group [Group II]** in **Paraquat model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Paraquat + Positive control group [Group III] showing lymphatic infiltration amounting to the histopathological score 1.00*

* **Scores:** [**0***: Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

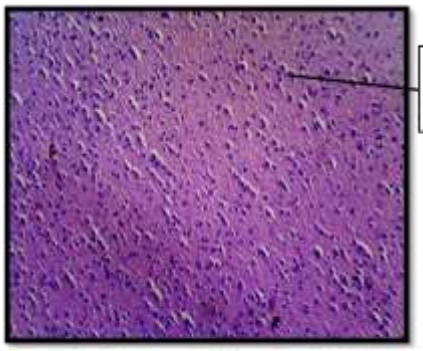
Figure 218: Section of rat brain [**Prefrontal cortex]** from **Paraquat + Levodopa & Benserazide [Group III]** in **Paraquat model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Paraquat + Captopril group [Group IV] showing mild neuronal loss amounting to the histopathological score 1.50*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

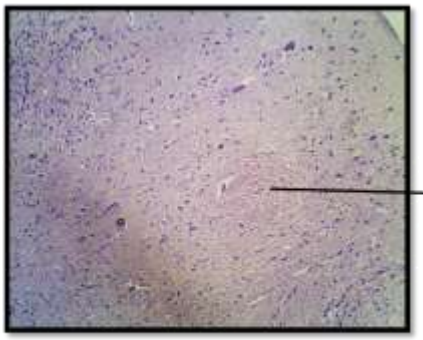
Figure 219: Section of rat brain [**Prefrontal cortex**] from **Paraquat + Captopril [Group IV]** in **Paraquat model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Paraquat + Perindopril group [Group V] showing gliosis and neuronal degeneration amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 220: Section of rat brain [**Prefrontal cortex**] from **Paraquat + Perindopril** [**Group V**] in **Paraquat model** (10x; H & E stained)



Section studied from the rat brain [Prefrontal cortex] of Paraquat + Losartan group [Group VI] showing mild edema with neuronsal loss amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

Figure 221: Section of rat brain [**Prefrontal cortex**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (10x; H & E stained)

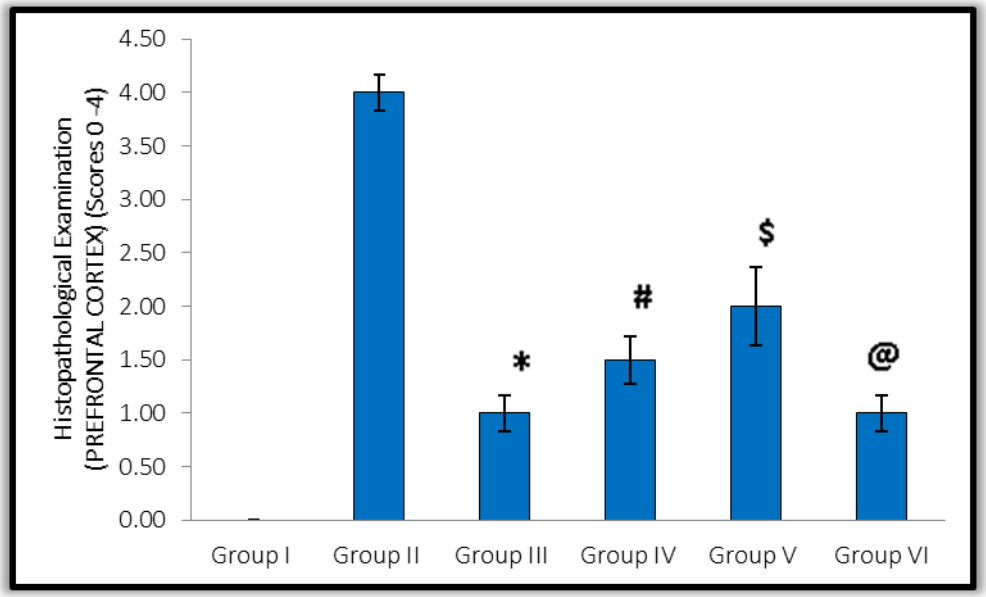
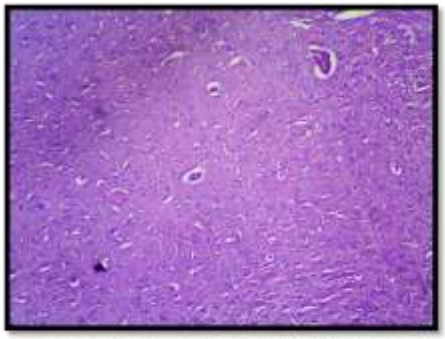


Figure 222: Bar diagram depicting the histopathological examination scores in Prefrontal cortex in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a statistically significant (P<0.05) increase in histopathological scores in the prefrontal cortex [cerebrum] as compared to the vehicle control group [Group I]. Moreover, it was witnessed that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) showed a statistically significant decrease in histopathological scores in the prefrontal cortex (P<0.05) when compared to the negative control group [Group II].

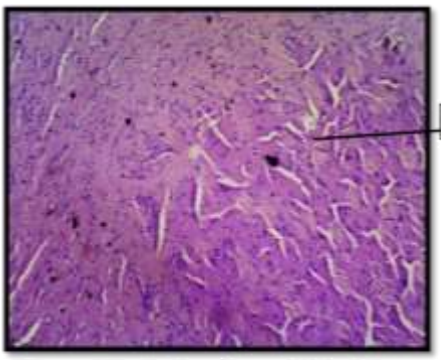
Microanatomical [Histopathological] examination of Corpus striatum [Basal nuclei] in Paraquat model



Section studied from the mouse brain [Corpus striatum] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

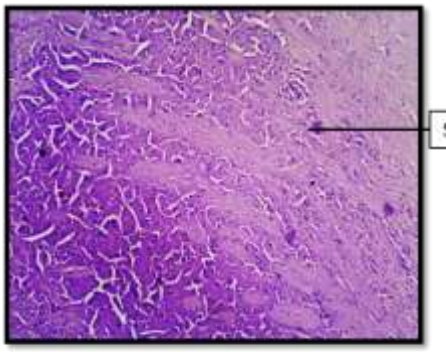
Figure 223: Section of mouse brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of Paraquat alone group [Group II] showing severe sclerosis amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

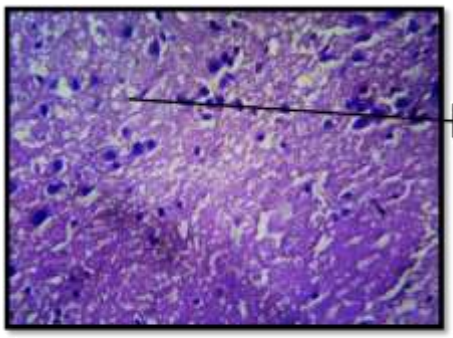
Figure 224: Section of mouse brain [**Corpus striatum**] from **Paraquat alone group [Group II]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Positive control group [Group III] showing mild sclerosis, degeneration and edema amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

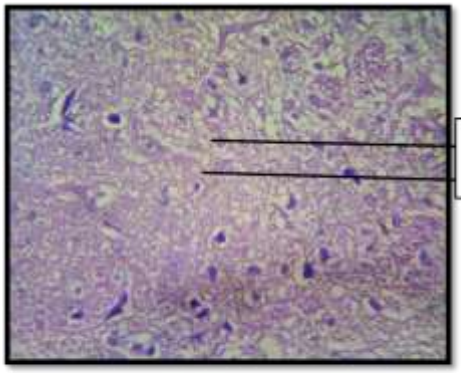
Figure 225: Section of mouse brain [**Corpus striatum**] from **Paraquat+ Levodopa & Benserazide [Group III]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Captopril group [Group IV] showing vacoulations amounting to the histopathological score .00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

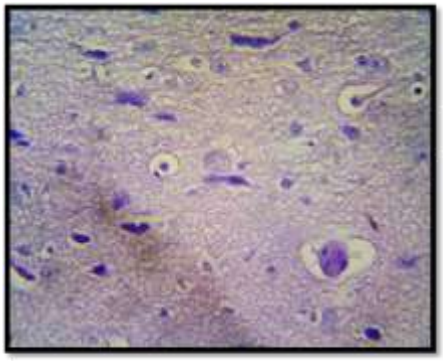
Figure 226: Section of mouse brain [**Corpus striatum**] from **Paraquat+ Captopril [Group IV]** in **Paraquat model** (40x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Perindopril group [Group V] showing degeneration and edema amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 227: Section of mouse brain [**Corpus striatum**] from **Paraquat + Perindopril [Group V]** in **Paraquat model** (40x; H & E stained)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Losartan group [Group VI] showing sclerosis and degeneration amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 228: Section of mouse brain [**Corpus striatum**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (40x; H & E stained)

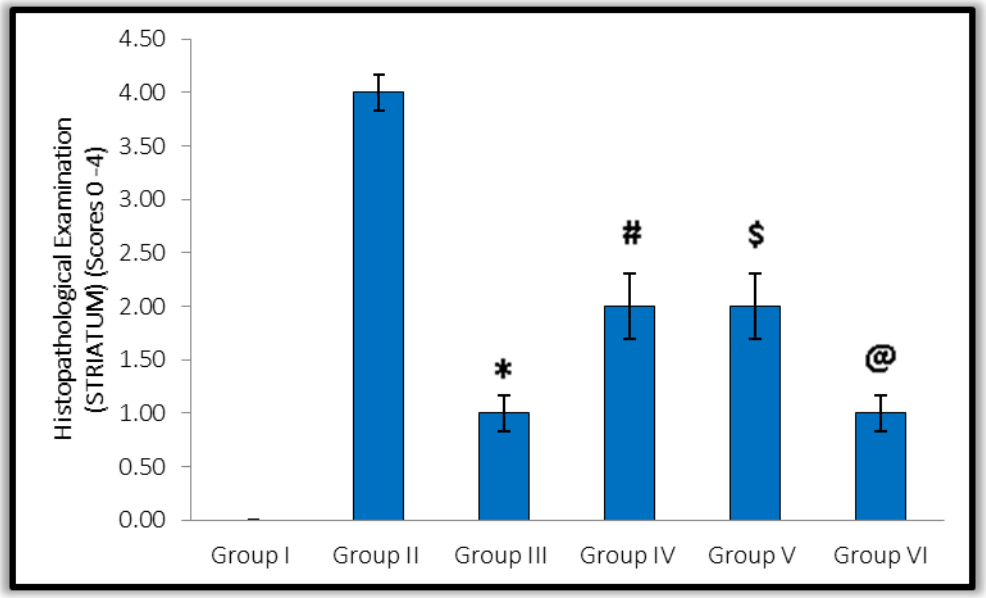
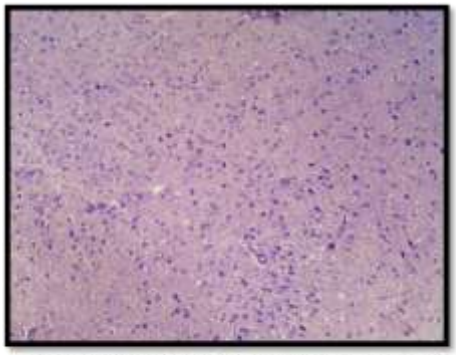


Figure 229: Bar diagram depicting the histopathological examination scores in the Corpus striatum in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a statistically significant (P<0.05) increase in histopathological scores in the corpus striatum [basal nuclei] when compared to the vehicle control group [Group I]. Besides that, it was noticed that the standard drug group [Group III] and the experimental drugs [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) resulted in a decrease in the histopathological scores of corpus striatum which was statistically significant when compared to the negative control group [Group II] (P<0.05).

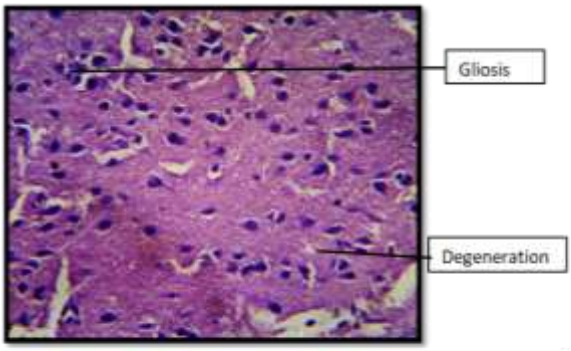
Microanatomical [Histopathological] examination of Hypothalamus in Paraquat model



Section studied from the mouse brain [Hypothalamus] of Vehicle control group [Group I] showing normal histology amounting to the histopathological score 0.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

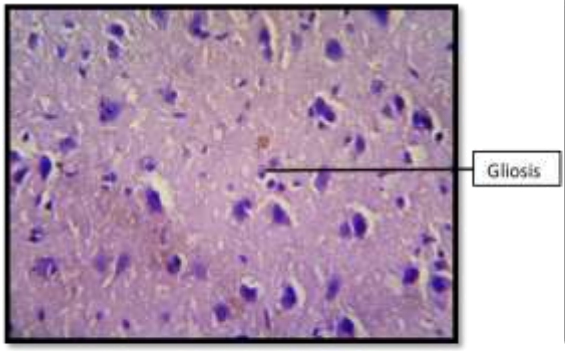
Figure 230 : Section of mouse brain **[Hypothalamus]** from **Vehicle control group [Group I]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of Paraquat alone group [Group II] showing severe gliosis and degeneration amounting to the histopathological score 4.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

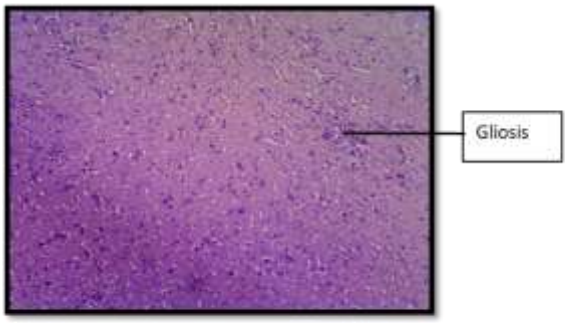
Figure 231: Section of mouse brain [**Hypothalamus**] from **Paraquat alone group [Group II]** in **Paraquat model** (40x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of Paraquat + Positive control group [Group III] showing mild gliosis amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1**: *>75% Healthy neuronal cells,* **2**: *50 - 74% Healthy neuronal cells,* **3**: *25 - 49% Healthy neuronal cells and* **4**: *<25% Healthy neuronal cells*]

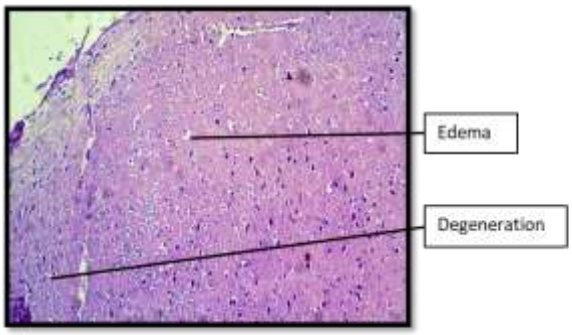
Figure 232: Section of mouse brain [**Hypothalamus**] from **Paraquat+ Levodopa & Benserazide [Group III]** in **Paraquat model** (40x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of Paraquat + Captopril group [Group IV] showing mild gliosis amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

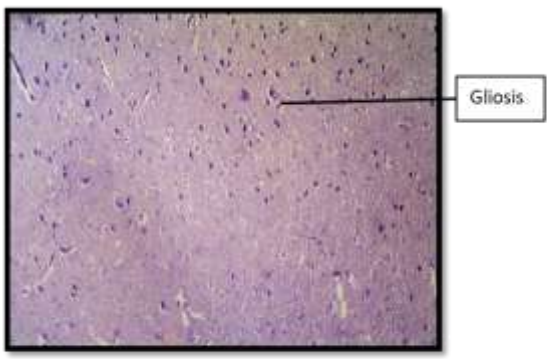
Figure 233: Section of mouse brain [**Hypothalamus**] from **Paraquat+ Captopril [Group IV]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Hypothalamus] from Paraquat + Perindopril group [Group V] showing degeneration and edema amounting to the histopathological score 2.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 234: Section of mouse brain [**Hypothalamus**] from **Paraquat + Perindopril [Group V]** in **Paraquat model** (10x; H & E stained)



Section studied from the mouse brain [Hypothalamus] of Paraquat + Losartan group [Group VI] showing Hippocampal gliosis amounting to the histopathological score 1.00*

* **Scores:** [**0**: *Normal healthy neuronal cells,* **1:** *>75% Healthy neuronal cells,* **2:** *50 - 74% Healthy neuronal cells,* **3:** *25 - 49% Healthy neuronal cells and* **4:** *<25% Healthy neuronal cells*]

Figure 235: Section of mouse brain [**Hypothalamus**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (10x; H & E stained)

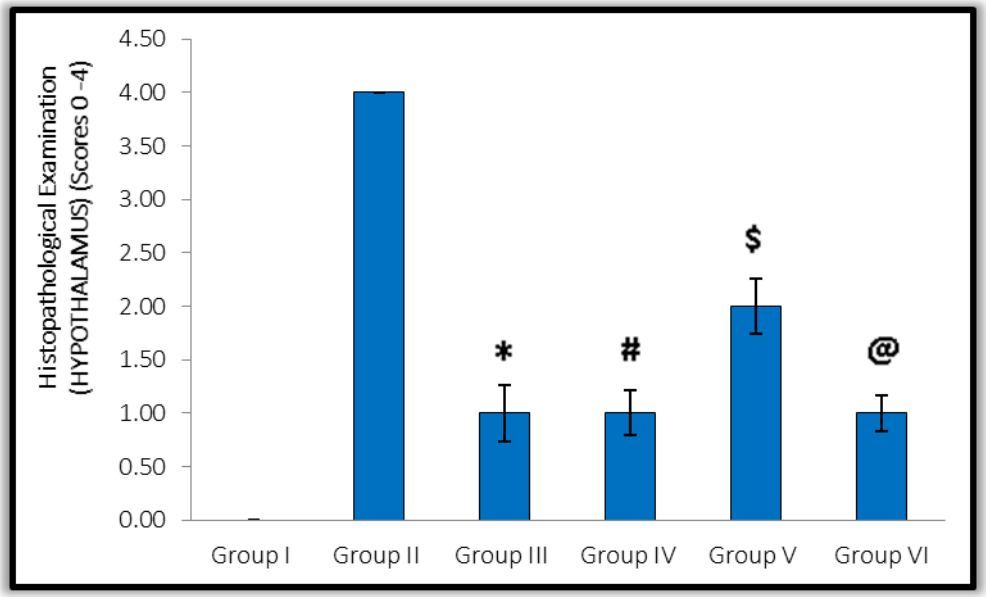


Figure 236: Bar diagram depicting the histopathological examination scores in the Hypothalamus in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The negative control group [Group II] had a statistically significant (P<0.05) increase in histopathological scores the hypothalamus when compared to the vehicle control group [Group I]. And furthermore, it was ascertained that the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) demonstrated a statistically significant decrease in the hypothalamic histopathological scores when compared to the negative control group [Group II] (P<0.05).

Table 28: Histopathological examination scores in Paraquat Model screening test in swiss albino mice

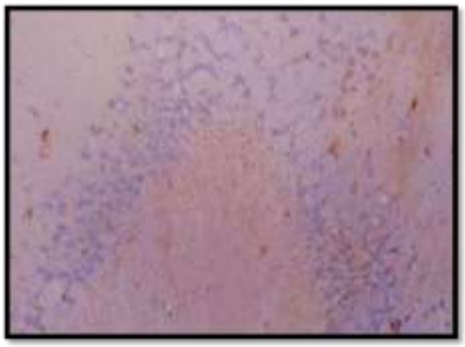
Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0-4)	0.00±0.00	4.00±0.21	1.00±0.21	2.00±0.37	2.00±0.31	1.00±0.21
2	Prefrontal Cortex (Scores 0-4)	0.00±0.00	4.00±0.17	1.00±0.17	1.50±0.22	2.00±0.37	1.00±0.17
3	Corpus striatum (Scores 0-4)	0.00±0.00	4.00±0.17	1.00±0.17	2.00±0.31	2.00±0.31	1.00±0.17
4	Hypothalamus (Scores 0-4)	0.00±0.00	4.00±0.00	1.00±0.26	1.00±0.21	2.00±0.26	1.00±0.17

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were significantly (P<0.05) raised in the negative control group [Group II] when compared to the vehicle control group [Group I]. Likewise, it was noticed that the standard drug group [Group III] and the experimental drugs [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) resulted in a statistically significant decrease in histopathological scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus when compared to the negative control group [Group II] (P<0.05).

EVALUATION OF IMMUNOHISTOCHEMISTRY IN PARAQUAT MODEL

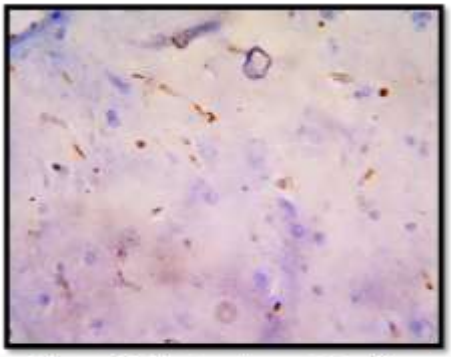
Immunohistochemistry of Hippocampus in various groups of Paraquat model



Section studied from the mouse brain [Hippocampus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

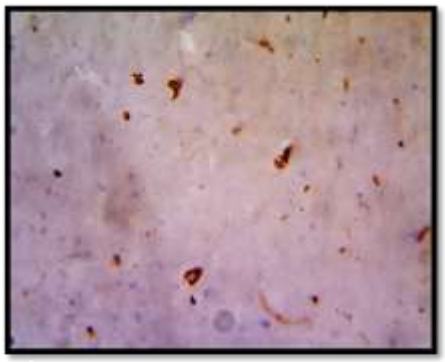
Figure 237: Section of mouse brain [**Hippocampus**] from **Vehicle control group [Group I]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] of Paraquat alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

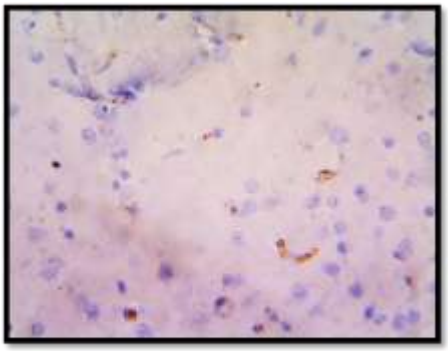
Figure 238: Section of mouse brain [**Hippocampus**] from **Paraquat alone group [Group II]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [hippocampus] of Paraquat+ Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

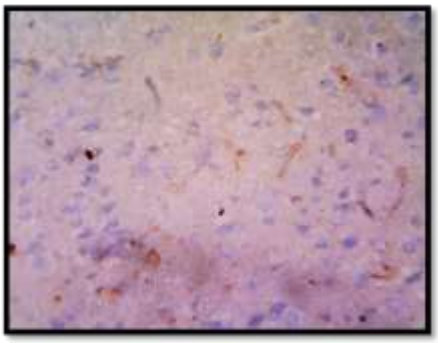
Figure 239: Section of mouse brain [**Hippocampus**] from **Paraquat + Levodopa & Benserazide [Group III]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [hippocampus] of Paraquat+ Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 2.5 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

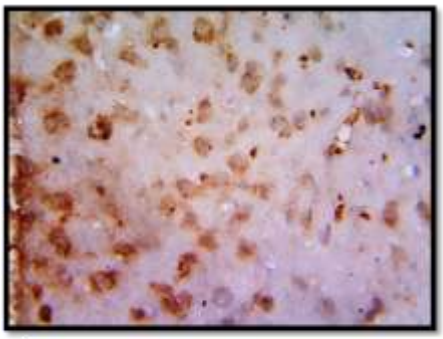
Figure 240: Section of mouse brain [**Hippocampus**] from **Paraquat + Captopril [Group IV]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] of Paraquat + Perindopril [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 2.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 241: Section of mouse brain [**Hippocampus**] from **Paraquat + Perindopril [Group V]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hippocampus] Paraquat + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 242: Section of mouse brain [**Hippocampus**] from **Paraquat + Losartan [Group VI] in Paraquat medeel** (40x; IHC Bcl-2)

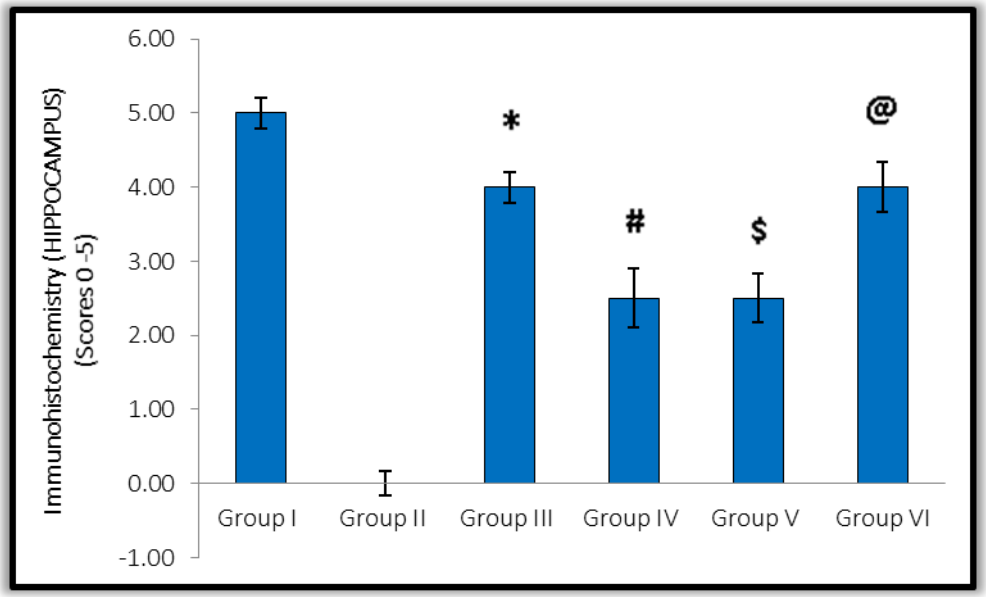
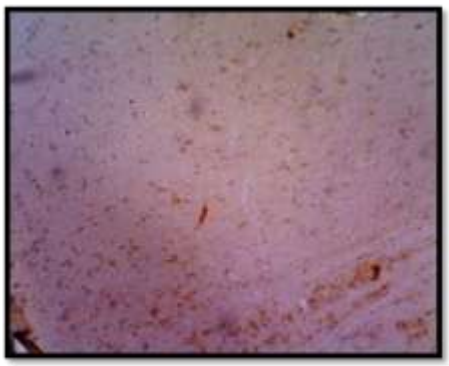


Figure 243: Bar diagram depicting the immunohistochemistry examination scores in Hippocampus in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores in the hippocampus were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Moreover, it was noted that both the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased IHC scores in the hippocampus, which was statistically significant when compared to the negative control group [Group II] (P<0.05).

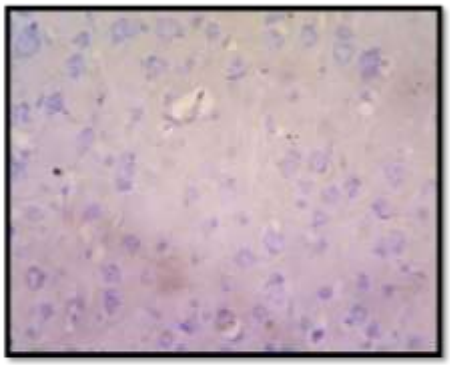
Immunohistochemistry of Prefronal cortex [Cerebrum] in various groups of Paraquat model



Section studied from the mouse brain [Prefrontal cortex] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

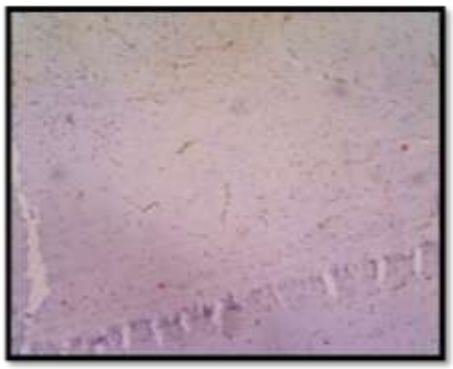
Figure 244: Section of mouse brain [**Prefrontal cortex**] from **Vehicle control group [Group I]** in **Paraquat model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of Paraquat alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

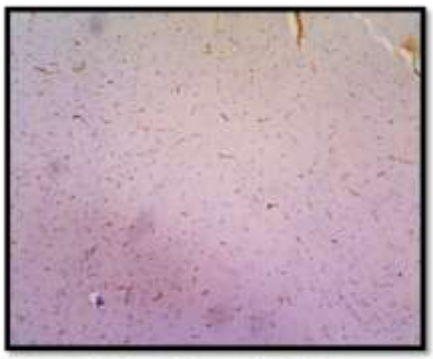
Figure 245: Section of mouse brain [**Prefrontal cortex**] from **Paraquat alone group [Group II]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of Paraquat + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0***: Nil/No neuroprotection,* **1:** *Plus*$^{+}$ *(Mild Neuroprotection),* **2:** *Plus*$^{++}$ *(Borderline Neuroprotection),* **3:** *Plus*$^{+++}$ *(Good Neuroprotection),* **4:** *Plus*$^{++++}$ *(Excellent/Normal Neuroprotection) and* **5:** *>Plus*$^{++++}$ *(Excellent/Normal Neuroprotection)*]

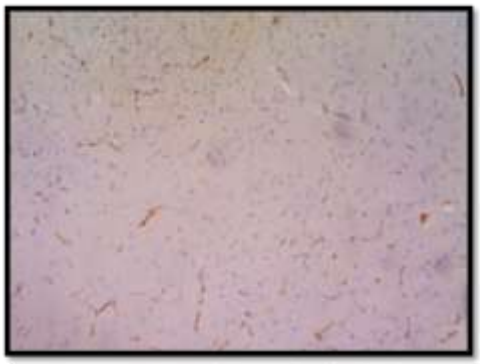
Figure 246: Section of mouse brain [**Prefrontal cortex**] from **Paraquat + Levodopa & Benserazide [Group III]** in **Paraquat model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of Paraquat + Perindopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

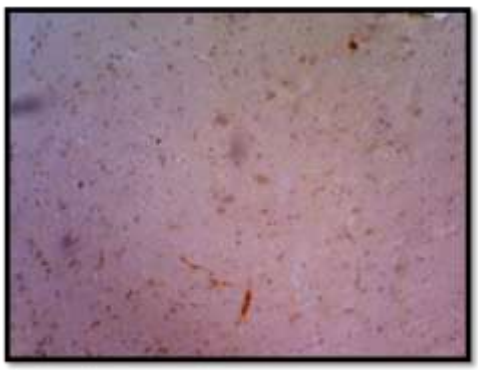
Figure 247: Section of mouse brain [**Prefrontal cortex**] from **Paraquat + Perindopril [Group IV]** in **Paraquat model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of **Paraquat + Perindopril** group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.50 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 248: Section of mouse brain [**Prefrontal cortex**] from **Paraquat + Perindopril [Group V]** in **Paraquat model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Prefrontal cortex] of **Paraquat +Losartan** group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 249 : Section of mouse brain [**Prefrontal cortex**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (10x; IHC Bcl-2)

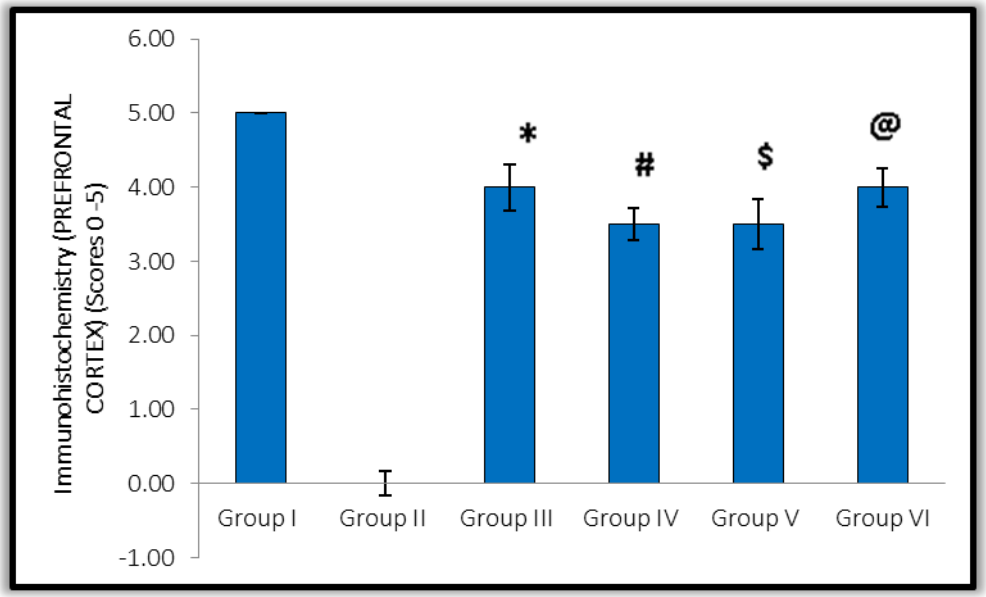
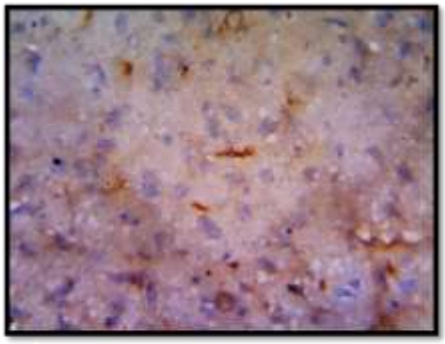


Figure 250: Bar diagram depicting the immunohistochemistry examination scores in Prefrontal cortex in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores in the prefrontal cortex [cerebrum] were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Subsequently, the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) exhibited a statistically significant increase in the IHC scores of the prefrontal cortex [cerebrum] when compared to the negative control group [Group II] (P<0.05).

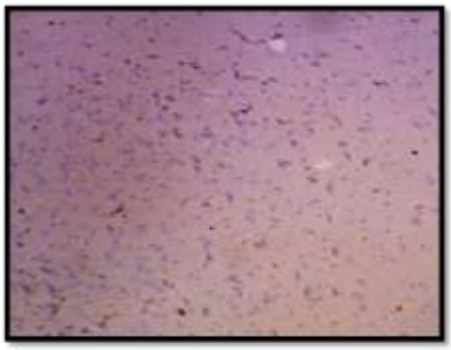
Immunohistochemistry of Corpus striatum [Basal nuclei] in various groups of Paraquat model



Section studied from the mouse brain [Corpus striatum] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

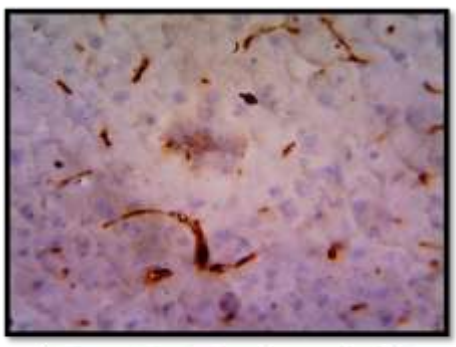
Figure 251: Section of mouse brain [**Corpus striatum**] from **Vehicle control group [Group I]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Paraquat alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**:*Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

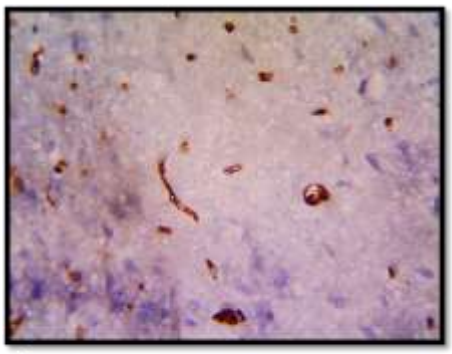
Figure 252: Section of mouse brain [**Corpus striatum**] from **Paraquat alone group [Group II]** in **Paraquat model** (10x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

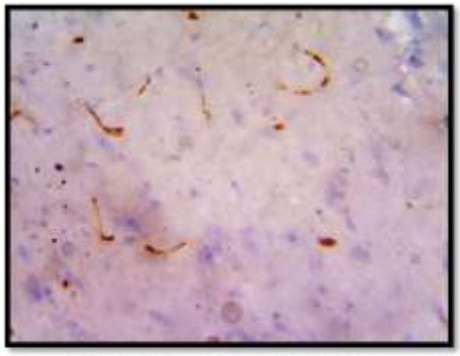
Figure 253: Section of mouse brain [**Corpus striatum**] from **Paraquat + Levodopa & Benserazide [Group III]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

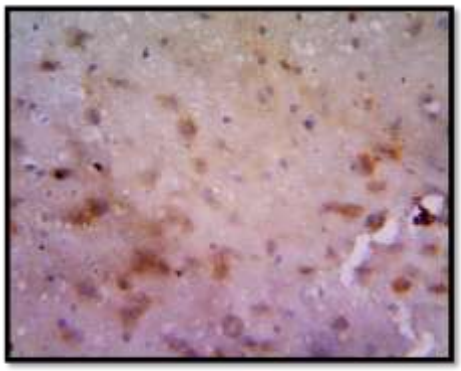
Figure 254: Section of mouse brain [**Corpus striatum**] from **Paraquat + Captopril [Group IV]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of Paraquat + Perindropil [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 255: Section of mouse brain [**Corpus striatum**] from **Paraquat + Perindropil [Group V]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Corpus striatum] of **Paraquat + Losartan** [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0***: Nil/No neuroprotection,* **1:** *Plus^{+} (Mild Neuroprotection),* **2:** *Plus^{++} (Borderline Neuroprotection),* **3:** *Plus^{+++} (Good Neuroprotection),* **4:** *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5:** *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 256: Section of mouse brain [**Corpus striatum**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (40x; IHC Bcl-2)

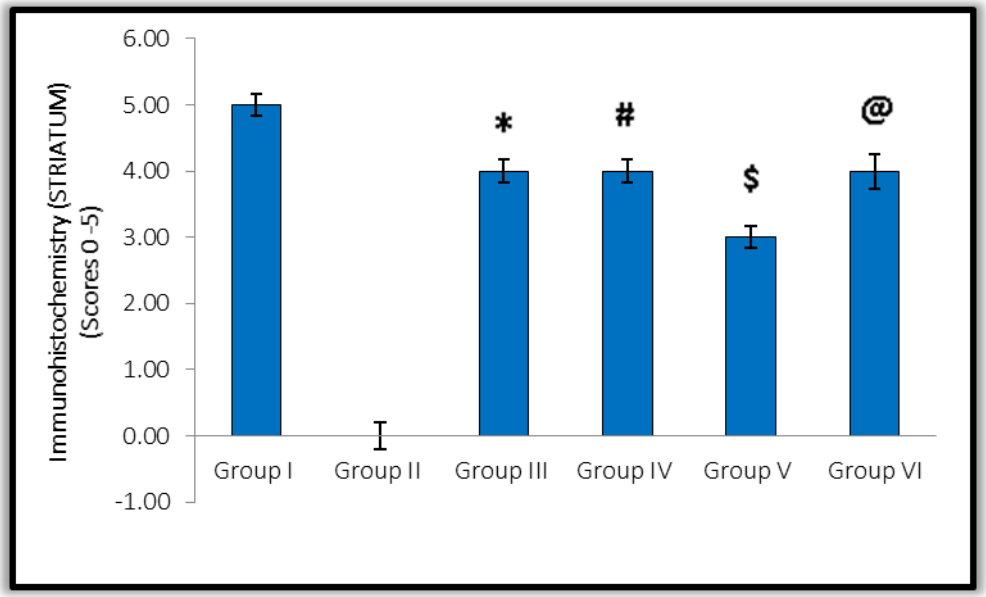
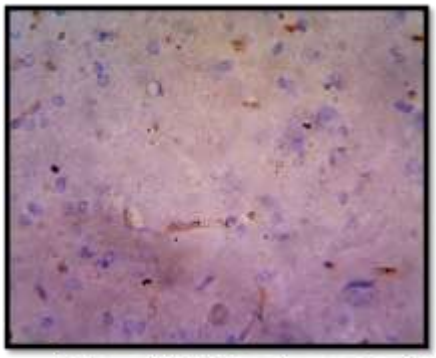


Figure 257: Bar diagram depicting the immunohistochemistry examination scores in the Corpus striatum in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

Immunohistochemistry scores in the corpus striatum [basal nuclei] were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Besides that, it was noticed that both the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased IHC scores in the corpus striatum, which was statistically significant when compared to the negative control group [Group II] (P<0.05).

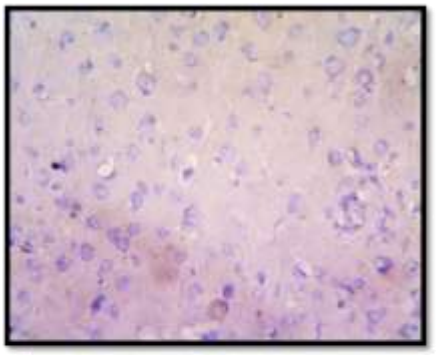
Immunohistochemistry of Hypothalamus in various groups of Paraquat model



Section studied from the mouse brain [Hypothalamus] of Vehicle control group [Group I] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 5.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

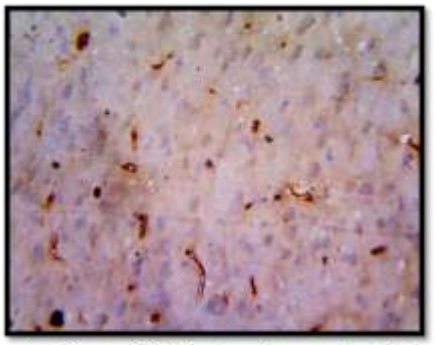
Figure 258: Section of mouse brain [**Hypothalamus**] from **Vehicle control group [Group I]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of Paraquat alone group [Group II] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 0.00 #

 # Scores: [**0**:*Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

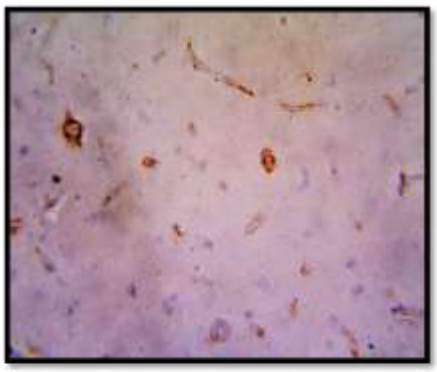
Figure 259: Section of mouse brain [**Hypothalamus**] from **Paraquat alone group [Group II]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of Paraquat+Positive control group [Group III] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

 # Scores: [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection)* and **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

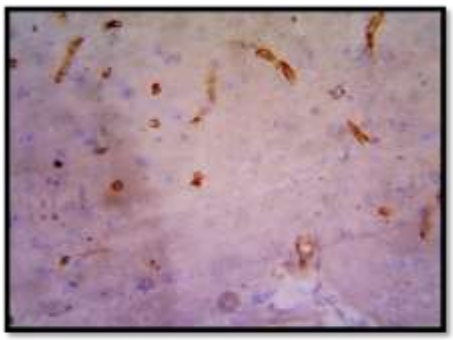
Figure 260: Section of mouse brain [**Hypothalamus**] from **Paraquat + Levodopa & Benserazide [Group III]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of Paraquat + Captopril group [Group IV] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

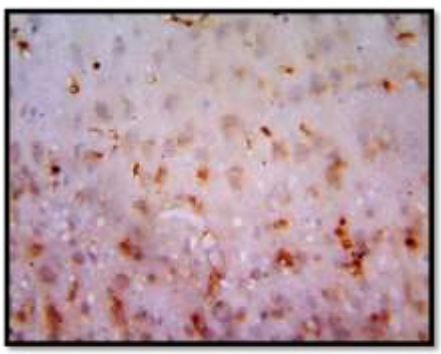
Figure 261: Section of mouse brain [**Hypothalamus**] from **Paraquat + Captopril [Group IV]** in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] **of Paraquat + Perindopril** group [Group V] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 3.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 262: Section of mouse brain **[Hypothalamus]** from **Paraquat + Perindopril** [Group V] in **Paraquat model** (40x; IHC Bcl-2)



Section studied from the mouse brain [Hypothalamus] of Paraquat + Losartan group [Group VI] showing immunoreactive 1+ with Bcl-2 amounting to IHC score 4.00 #

**Scores:** [**0**: *Nil/No neuroprotection,* **1**: *Plus^{+} (Mild Neuroprotection),* **2**: *Plus^{++} (Borderline Neuroprotection),* **3**: *Plus^{+++} (Good Neuroprotection),* **4**: *Plus^{++++} (Excellent/Normal Neuroprotection) and* **5**: *>Plus^{++++} (Excellent/Normal Neuroprotection)*]

Figure 263: Section of mouse brain [**Hypothalamus**] from **Paraquat + Losartan [Group VI]** in **Paraquat model** (40x; IHC Bcl-2)

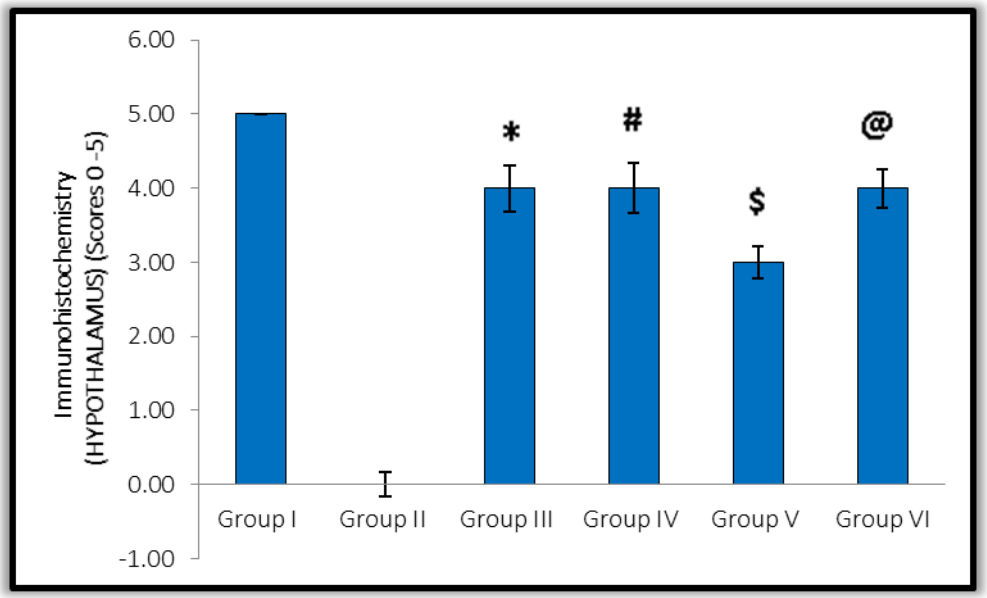


Figure 264: Bar diagram depicting the immunohistochemistry examination scores in Hypothalamus in Paraquat Model screening test in swiss albino mice

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

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The immunohistochemistry scores in the hypothalamus were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Likewise, it was noted that both the standard drug group [Group III] and the experimental groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased IHC scores in the hypothalamus, which was statistically significant when compared to the negative control group [Group II] (P<0.05).

Table 29: Immunohistochemistry examination scores in Paraquat Model screening test in swiss albino mice

Sl. No.	Parameters	Group I	Group II	Group III*	Group IV#	Group V$	Group VI@
1	Hippocampus (Scores 0-5)	5.00±0.21	0.00±0.17	4.00±0.21	2.50±0.40	2.50±0.33	4.00±0.34
2	Prefrontal Cortex (Scores 0-5)	5.00±0.00	0.00±0.17	4.00±0.31	3.50±0.22	3.50±0.33	4.00±0.26
3	Corpus striatum (Scores 0 -5)	5.00±0.17	0.00±0.21	4.00±0.17	4.00±0.17	3.00±0.17	4.00±0.26
4	Hypothalamus (Scores 0-5)	5.00±0.00	0.00±0.17	4.00±0.31	4.00±0.34	3.00±0.21	4.00±0.26

Data are represented as Mean ± SE; n = 6 in each Group; *P < 0.05, #P < 0.05, $P < 0.05 and @P < 0.05, when compared to Group II.

The immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus were significantly (P<0.05) lower in the negative control group [Group II] than in the vehicle control group [Group I]. Moreover, it was evidenced that both the standard drug group [Group III] and the experimental drug groups [Group IV, Group V and Group VI] (Captopril, Perindopril and Losartan) increased immunohistochemistry scores in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus, which was statistically significant when compared to the negative control group [Group II] (P<0.05).

DISCUSSION

DISCUSSION

With increased emphasis on the brain renin angiotensin system and the neuroinflammatory processes accelerating the nigrostriatal dopaminergic neuronal loss in the midbrain, there is a renewed interest in identifying a novel therapeutic agent addressing the underlying pathological process. MPTP model, being one of the most popular and an easy way to reproduce parkinsonism like theme with the nigrostriatal dopaminergic neuronal loss, had been widely used by many researchers to prove the beneficial role of experimental drugs.

In the present study, suboptimal dose of MPTP (25 mg/kg) had been repeatedly given intraperitoneally to induce the parkinsonism. Newer researchers have advocated administration of MPTP selectively into the midbrain. However, for the want of expertise in such intracranial injections, such complex procedures were not undertaken. Both the experimental groups received intraperitoneal injections of levodopa and perindopril for seven days. It was postulated that the combined administration of MPTP with perindopril should influence the neurodegenerative process of the toxin. This resulted in less severe induction of the parkinsonism.

After the epidemiological evidences of pesticide use as risk factor in the development of PD, and it had been proved that, few of these compounds cause nigrostriatal dopaminergic neuronal loss. This led to the development of rotenone and paraquat animal models.[237] These two affect the complex-I component of respiratory chain in mitochondria.[238] However, in the rotenone model, the extent of motor manifestations and the dopaminergic neuronal loss is highly variable. This leads to a very high numbers of animals in each study group.[160] Other than this, rotenone induces extensive extra-nigral cell death.[161] Such neuronal loss is not consistent with

PD. With all this limitations also, rotenone model has been successfully shown beneficial effects of many drugs including L-dopa.[157, 158]

Even though, paraquat also induces the nigrostriatal dopaminergic neuronal cell death in much like rotenone by influencing mitochondrial complex-I, considering its superior animal model as, it was considered at least by few as a gold standard animal model[239]. However, there is a voice of dissent for this.[240] Many researchers have used paraquat model along with a fungicide, maneb with variable success.[173,176] In the present study, paraquat model in the mice were used for evaluating the drugs. However, the model is effective in rats and mice alike.[241]

Validity of these models in assessing the symptomatic therapies is questioned recently. There is a notion that even though many of the drugs and other molecules that were highly promising in the animal models had failed to follow through phase 1 and II clinical trials with very less demonstrable efficacy.[242] Examples included NS2330, BTS 74 398 and brasofensine (non-selective monoamine uptake inhibitors) that were highly effective in rodent MPTP model and 6-OHDA model. However, when these drugs were evaluated in the clinical trials, there was very limited functional improvement and the higher incidence of side effects.[243, 244] This led to a call by few researchers for a paradigm shift in the entire evaluation pattern of the drugs for the PD therapy.[245]

With this background, it may be said that, there is no single animal model that effectively assess the experimental drug for PD therapy. This substantiated our efforts of studying the drugs in question in three different models, with both the rats and the mice.

Neuro-behavioural analysis

In the present study, behavioural effects were studied by spontaneous locomotor activity which was evident through actophotometer and with rotarod test. These two methods are well established in means of measuring overall behavioural changes in the rodent model. Similar improvement in the motor actions were noted in rats treated with pseudoginsenoside,[246] candesartan[110] and azilsartan[247] enalapril and moexipril[248] in MPTP model.

Actophotometer measurement of spontaneous locomotor activity gives appropriate estimation of overall motor functions. Counts per ten minutes were used as an index of locomotor activity. In the rotenone model, rats treated with perindopril and losartan showed a significant improvement in the motor co-ordination. In the MPTP model and in paraquat model, all the groups showed a significant lower motor co-ordination. This implied that only perindopril and losartan in rotenone model had a significant positive influence on the spontaneous locomotor activity.

Similar improvement in the spontaneous locomotor activity was observed after the administration of Juniperus communis (in chlorpromazine induced rat model,[249] Trigonella foenum-graecum seed extract (in 6-OHDA induced unilateral PD in rats)[250] and in Artemisia flowers (in chlorpromazine induced rat model)[251]and curcumin and derivatives (in 6-OHDA induced rat model).[252] In addition, the actophotometer was recorded a better motor activity in MPTP rats pre-treated with ceftriaxone in rescuing the dopaminergic neuronal loss.[253]

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. Rotarod test is a commonly used test to evaluate the beneficial effects of the test drugs and molecules.[254] There are various standardizations and formulae to estimate the

beneficial effects in the laboratory animals. In the present study, all the groups in three models significantly increased the duration of rotarod latency to fall. Perindopril had shown a significant improvement of motor co-ordination in rotenone and paraquat model. Administration of perindopril and losartan had resulted in significantly improved motor co-ordination in all three PD models.

In the present study, captopril had showed a significant improvement in the motor activity and the co-ordination (as evidenced through rotarod test and actophotometer) in paraquat model. Perindopril had shown significant improvement in the motor activity and the co-ordination (as evidenced through rotarod test and actophotometer) in rotenone and MPTP models. Perindopril had shown a significant improvement in the motor activity and the co-ordination (as evidenced through rotarod test and actophotometer) in rotenone, MPTP and paraquat models.

Similar improved motor co-ordination preventing the rat or mice from falling from the rotarod had been recorded after the administration of Dendropanax morbiferus leaves (MPTP model),[255] Apium graveolens (MPTP model),[256] Juniperus communis (CPZ model),[249] caffeine (rotenone model),[257] and with many other chine herbal medicines.[254,255] Performance of animal on the rotarod test may be influenced by all the factors affecting the motor system. Previous exposure to this test was the most important factor influencing the outcome. Rodents habituated to the balancing on the rotating base at slower revolutions were found to have longer latency to the fall duration and the longer distance covered. However, this test had fulfilled all the essential criteria for the validation in the pre-clinical evaluation of the drugs.[258] Therefore, from the present results, we could convincingly concluded that captopril, perindopril and losartan significantly improved the motor co-ordination in the rodent models in this study.

Improvement in the muscle strength is reported after the administration of caffeine[257] in rotenone induced rat model of PD and isolongifolene in MPTP induced model of PD.[259] In addition to the fore limb hang test, forepaw wire test also checks the strength of muscles. As the pathogenesis of loss of power is different from the general akinesia or dyskinesia, evaluation of this parameter suggested the effect of the experimental drug on the muscle strength.

The hole board test was used to examine exploratory behaviour. The open field test is another behaviour analytic method for assessing exploratory behaviour. This test is carried out in a brightly illuminated environment, and the animal was not given a choice. Furthermore, open field tests do not distinguish between locomotion and exploration. Hole board test instrument, on the other hand, excels at detecting both exploration and locomotion. The head-dipping, rearing, and locomotion of the animal on the platform are three crucial activities. This method is advanced by the use of infrared rays. In the present study, captopril had shown a significant improvement in the exploratory behaviour (as evidenced through the hole board test) in rotenone and paraquat models. Perindopril had shown a significant improvement in the exploratory behaviour (as evidenced through the hole board test) in rotenone and paraquat models. Losartan had shown a significant improvement in the exploratory behaviour (as evidenced through hole board test) in rotenone, MPTP and paraquat models. Caffeine, [257] arbutin,[260] dasatinib, and resveratrol[261] had all been satisfactorily tested using the hole board test. However, a decreased head dipping has been reported in many studies, especially on repeated exposures of animals to the test. The results of head dipping test may also be considered as a part of neophilia than the exploratory locomotive response. There are reports considering these head dipping

behaviour as an escape response, that decreases as the exposed rodents becomes less fearful.[222]

In the present study, captopril had shown a significant anti-depression effects (as evidenced through tail suspension test and forced swim test) in rotenone, MPTP and paraquat models. Perindopril had shown a significant anti-depression effects (as evidenced through tail suspension test and forced swim test) in rotenone, MPTP and paraquat models. Losartan had shown a significant anti-depression effects (as evidenced through tail suspension test and forced swim test) in rotenone, MPTP and paraquat models.

Ipramine,[262] fluoxetine, reboxetine, moclobemide[263] and nitroindazole[264] had all been satisfactorily evaluated using the forced swimming test. Serotonin-selective reuptake inhibitors (fluoxetine, certraline, sertraline) had been shown to promote swimming behaviour. Climbing behaviour was improved by drugs that boost norepinephrine and dopamine.[265] From this present study, it was evident that captropril, perindopril and losartan improved the depression status by decreasing immobility duration in forced swimming test by influencing the serotonin levels; however, the exact mechanism could not be ascertained with this study design.

The forced swimming test is most typically used to assess behavioural despair. The rodent's immobility time spent on the water is used to assess depression-like behaviour. This test is popular in both traditional and modified forms.[266] Activity scoring (swimming or climbing) offers a measure of the rodent's response to drowning in a modified version of this test.[267] Many drugs had shown to improve the immobility duration spent in this test and it is successfully used to pre-clinically assess the antidepressant efficacy of these drugs.

In an elevated plus maze test, the number of entries into the open arm, the number of entries into the closed arm, and the time spent in the open arm are all indicators of the animal's anxiety. According to some studies, even a partial dopaminergic degeneration with noradrenergic and serotonergic alterations causes emotional and cognitive impairments during the early stages of PD.[268] Similarly, the present study conclusively proved that captopril had shown a significant anti-anxiety effects (as evidenced through elevated plus maze test) in rotenone, MPTP and paraquat models. Perindopril had shown a significant anti-anxiety effects (as evidenced through elevated plus maze test) in rotenone, MPTP and paraquat models. Losartan had also shown a significant anti-anxiety effects only (as evidenced through elevated plus maze test) in MPTP and paraquat models.

In the present study, all the three test drugs showed a significant decrease in the immobility time in all the three animal models in tail suspension test. This meant that captopril, perindopril, and losartan had a positive impact on the rotenone, MPTP, and paraquat models' emotional and cognitive deficiencies. In rodents models, the application of reserpine (an antihypertensive, adrenergic uptake inhibitor),[269] Artemisia,[251] caffeine,[257] and Juniperus had similar positive benefits (coniferous trees and shrubs).[249] From the present study, we concluded that the three test drugs that are influencing the brain angiotensin system had shown a significant non-motor improvement (as evidenced through forced swimming test, tail suspension test, hole board test and elevated plus maze test) than symptomatic motor improvement (as evidenced through rota rod test and spontaneous motor activity). Thus, we broadly concluded that captopril, perindopril and losartan have significantly more beneficial in the non-motor therapy of PD (at least in rodent model).

Oxidative stress markers

Various drugs and plant extracts had been used to assess the role of oxidative stress in neuroinflammatory processes.[270] In this process, the majority of studies suggest that free radicals interact directly with microglia, astrocytes, and neurons.[271] In the present study, captopril had shown a significantly lower levels of LPO and therefore, a higher oxidative protection in rotenone and paraquat models. Perindopril also had shown a significantly lower levels of LPO and therefore, higher oxidative protection in all the three animal models- rotenone, MPTP and paraquat. Losartan too had shown a significantly lower levels of LPO and therefore, higher oxidative protection in rotenone, MPTP and paraquat models. With these results, we convincingly concluded that captopril, perindopril and losartan had shown a significantly decreased oxidative stress levels in the rotenone and paraquat model.

Lipid peroxidase levels and myeloperoxidase were significantly decreased in all the test drug groups of rotenone, MPTP and paraquat models. This showed that the test drugs effectively and significantly scavenged free radical species during and after toxin-induced neuronal injury in the rodents. In the present study, captopril had shown a significantly higher levels of catalase and therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models. Perindopril had shown a significantly higher levels of catalase and therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models. Losartan also had shown a significantly higher levels of catalase and therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models.

In the present study, captopril had shown a significantly higher levels of SOD and therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models. Perindopril had shown a significantly higher levels of SOD and

therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models. Losartan had shown a significantly higher levels of SOD and therefore, greater protection against the oxidative injury in rotenone, MPTP and paraquat models.

"Similar neuroprotective role of some of the herbal derivatives from ginger (curcumin), ginseng (ginsenoside) and polygonum cuspidatum (resveratrol) has been reported.[272] Herbal extracts like moutan cortex, Angelica dahurica root, and bupleurum root also exerts neuroprotective action in PD.[273] Many flavonoids are proposed to exhibit the neuroprotective actions primarily through anti-oxidant mechanisms.[274] Apart from these, Artemisia,[251] isolongifolene[259] and caffeine[257] and many other drugs have shown significant therapeutic roles in PD."

Even though the current study suggests that captopril, perindopril, and losartan have a major neuroprotective role with the parameters evaluated, more research specifically quantifying their roles will be needed to prove this attribute conclusively.

Neurotransmitter evaluation

Serotonin

Although dopamine is implicated in all the clinical manifestations of the parkinsonism, there is a growing evidence to support steady, sustained and the non-linear loss of serotonergic neurons that adds to the motor and non-motor symptoms.[186-189] This challenges the age-old dopamine only theory of parkinsonism. Among all the serotonin receptors, 5-HT_{2A} receptors are implicated in the cognitive and executive functions of the neurodegenerative disorders including alzheimer's disease and parkinsonism.[275]

In the MPTP and rotenone models, serotonin levels were dramatically lowered in all groups. In the current study, however, serotonin levels in the levodopa,

perindopril, and losartan groups were considerably greater than the control group in the paraquat model. Highest levels were noted in the rodents receiving the levodopa. However, the significantly lower levels were documented in captopril. Model wise, following observations were made - captopril had shown a significant decrease in the serotonergic damage (as evidenced through the increased serotonin levels) in the rotenone, MPTP and paraquat models; Perindopril had shown a significant decrease in the serotonergic damage (as evidenced through the increased serotonin levels) in the rotenone, MPTP and paraquat models; Losartan had shown a significant decrease in the serotonergic damage (as evidenced through the increased serotonin levels) in the rotenone, MPTP and paraquat models.

This, together with the fact that bradykinesia was reduced, suggested that serotonergic pathways play a role in the overall effects of levodopa, captopril, perindopril, and losartan. The particular mechanism of action, however, could not be determined by measuring total serotonin. According to a study on levodopa-induced dyskinesia in rats, there appears to be a complex interplay between serotonergic neurons and dopamine release.[190, 276]

As there is a complex multitude of subtypes of 5-HT receptors in the brain, pre-synaptic stimulation of one of the receptor subtypes may be postulated for the noted increase in the serotonin levels in the paraquat model, especially with the levodopa administration. Specific receptor estimation by the autoradiography shall provide a clear understanding of such a variations in the serotonin overall levels. We, therefore, did not ascribe much importance to this noted deviation from the previous studies.

Dopamine

The loss of dopaminergic neurons in the substantia nigra pars compacta is a characteristic of PD. The efficacy of animal models is determined on the degree to which they cause neuronal death and dopamine depletion. There are many methods to quantify the neuronal loss. One of the broader methods is to quantify the dopamine levels in the brain. In the present study, dopamine levels in MPTP and paraquat groups where PD was induced and had not received any drugs [negative control groups] were significantly lower than the vehicle control groups. Rotenone induced PD rats did not show a significant decrease in the dopamine. Recorded dopamine levels in the brain homogenates from captopril, perindopril and losartan groups of rotenone and MPTP models showed a significant increase in the dopamine level. This may be attributed to the neuroprotection and less severe damage of dopaminergic neurons among these animals. However, the paraquat model had not shown any significant changes in the dopamine level.

Similar improvement in the dopamine levels were postulated to be due to the neuroprotective effects of the test drugs had been reported after the administration of biochanin A(in MPTP model),[277] adenosine A receptor blockers (rotenone model)[278] and catechin (6-OHDA model).[279] There are many reports concentrating on the dopamine levels in the specific regions of the brain and has shown that co-administration of artemisi,[251] Juniperus[249] and many Chinese herbal preparation[254, 255] resulted in improved dopamine levels. This concluded that these interventions results are in favour of lesser neuronal damage. It may also be a paradoxical initial transitory increase in the dopamine level as evidenced by a report in the monkey MPTP model.[280]

GABA

GABA is an important inhibitory neurotransmitter in the brain. There is a growing evidence to show that along with calcium, GABA and synuclein plays a crucial and the decisive role in local inflammation leading to the neuronal destruction in the degenerative disorders.[198] GABA - collapse hypothesis is the forerunner in the current understanding of the neuronal mechanism underlying the dopaminergic neuronal damage in PD[197]

In the present study, GABA levels were significantly lower in all the animals of paraquat model and in the captopril group of rotenone model. GABA being an important inhibitory neurotransmitter, lower levels of GABA with an increase in the calcium has been the proven pathway of neuronal destruction in not just in PD but also in alzheimer's disease.[281] A decrease in the GABA inhibitory activity leads to differential co-activation of agonist and antagonist motor components in the corpus striatum.[282]

Contrary to this observation, we noted a significantly increased level of GABA in all the groups of MPTP model. GABA when released into the extracellular space, it is taken up into the neurons and glial cells. In addition to this, GABA is taken up differentially into the various neuronal components that are specific to the brain region and to the specific functions. Astrocytes also play a major role in GABA metabolism.[283] The observed increase of GABA in all the groups of rotenone model as compared to the rest of the models might be attributed to the differential metabolism of GABA through astrocytes.[284] To support this, a recent study evaluating neurotransmitter levels using single-voxel GABA-edited spectra through magnetic resonance imaging and spectroscopy (MRI/MRS) method concluded that GABA levels are more on the dominant side of basal ganglia in PD patients.[285]

Glutamate

The axial symptoms of PD including imbalance, impaired posture, problems in speech, difficulty in swallowing, freezing of gait and axial rigidity typically show less response to the dopamine directed therapy. There may be an involvement of other neurotransmitters, especially glutamate and GABA in the pathogenesis of these symptoms. The neuronal mechanisms are listed under GABA section. In addition, recent studies had shown that eight proteins implicated in PD have functional roles in astrocyte biology highlighting the influences of glutamate-GABA-glutamate recycling system.[197]

In the present study, glutamate levels were significantly lower in all the groups of rotenone and in most of the groups of MPTP and paraquat models. This lower glutamate levels were in-line with many studies and also goes according to GABA – collapse theory that lower glutamate-GABA levels lead to higher inflammation and resultant is the neuronal loss in the corpus striatum.[197] The glutamate levels were observed in contrary to this in the mice induced PD with MPTP and mice induced with paraquat without receiving any pre-tretment with test drugs. A complex interplay of glutamate-GABA of the specific areas of the brain in the related astrocytes might have resulted in such a contrarian increase in the glutamate. Regional evaluation of the glutamate in these rodent models shall bring more clarity in such situations.

Acetylcholine

As explained in the previous section, acetylcholine plays a crucial role in the pathogenesis of certain manifestations of PD, particularly, cognitive impairment, dyskinesia and sleep changes.[202,203] However, there are conflicting animal model studies to support this. Few reports have exclusively stressed on the pedunculopontine

nuclear lesions resulting in such a cognitive symptoms; other researchers failed to highlight any such a lesions in the animal models.

In the present study, significantly lower level of acetylcholine was noted in all the animals across all the three animal models. However, significantly increased acetylcholine levels were found in MPTP only group and paraquat only group without the pre-treatment of test drug [negative control groups]. With increasing dichotomy of the reported literature about the role of acetylcholine in the animal models, our study too fitted into this scenario of noting different levels of acetylcholine in the rodent brain homogenate samples of the toxin models.

Histopathological [Microanatomical] changes in rodent model of PD

From the days of exclusive importance to dopaminergic neuronal loss in the substantia nigra region, interest in PD histological changes has moved to a regional astrocyte and glial changes, and to the histological changes in the various parts of nervous system including enteric nervous system (myenteric plexus), vagus nerve and olfactory nerve. PD is no more restricted to substantia nigra culminating as a motor symptomatic triad dominated by rigidity, akinesia and tremors. Consideration of Hippocampus, Prefrontal cortex (Cerebrum), Corpus striatum (Basal nuclei) and Hypothalamus provides a meaningful insight into not only the non-motor symptoms but also to the variations and the differential manifestations of motor symptoms.[206, 208, 213]

In the present study, changes varying from a subtle glial pale body formation to the striking inclusion bodies, neuronal loss were recorded. Maximum cellular changes with neuronal loss had been recorded in the MPTP model and paraquat model of rodents that were not pre-treated with any test drugs [negative control groups]. This proved the toxic effects of these compounds yet again. There are

innumerable studies in the past providing convincing evidences for the dopaminergic neuronal loss after injection or oral administration of these toxins.[164,165,173,176,193,239,241] In addition, we also observed edema in the prefrontal cortex of cerebrum and corpus striatum after MPTP injection. It also had resulted in vacuolations, hyperchromatic and shrunken cells in the prefrontal cortex of cerebrum. Similarly, in the paraquat model, along with cerebral edema, many immature granular cells with pale nucleus were seen and the vacuolations were evident around the cells in the cerebrum. And also hyperchromatic pyknotic nucleated neurons and edema in the cerebrum along with immature cells were observed.

Overall, from the present study, it was observed that, captopril had not shown any significant edema or vacuolated cells in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus of rotenone, MPTP and paraquat models. Similarly, perindopril also had not shown any significant edema or vacuolated cells in the hippocampus, prefrontal cortex, corpus striatum and hypothalamus of rotenone, MPTP and paraquat models. The losartan also had not shown any significant edema or vacuolated cells in the brain sections that were studied in rotenone, MPTP and paraquat models.

The brain sections of captopril, perindopril and losartan treated rodents showed a histopathological lesions like mild vacuolations, mild edema (in hippocampus, Prefrontal cortex, Corpus striatum, Hypothalamus), apoptic cells, astrocyte variations, pale granular cells, glial pale bodies (in cerebrum and corpus striatum), hyperchromatic and pyknotic nuclei in the specific regions of the brain. But, the overall neuronal architecture were not altered significantly in comparison to the rodent brains that were not pre-treated with the test drugs. The histological picture was in line with the neuro behaviour effects, the oxidative stress markers levels,

neurotransmitters levels and inflammatory marker in protecting the neurons against the toxins used to induce the PD.

Researchers had shown similar histological changes with the use of Ficus religiosa leaves extract. In this study of them, the documented histological changes were increased intracellular space, infiltration of neutrophils, decreased density of cells and neuronal cell death.[227] In our study, we had not documented any increased cellular sizes. Many of the neurons across several regions of the brain were shrunken and darkly stained as explained earlier.

Similar histological changes as reported in this study were found by researchers evaluating neuroprotective role of salsolinol. They had documented the marked improvement in the histo-architecture of rat brain after administration of different doses of salsolinol.[286] However, our study design did not inferred such a results of improvement or normalization of histological architecture.

Researchers have recorded similar neuronal loss and histological changes in a study evaluating the effects of plant extracts of Paeoniaemodi wall in 6-OHDA rodent model.[287] Here, the researchers had used an arbitrary grading system to objectively evaluate the histological changes in the rat brain sections. This grading system is not standardized. It may lead to difficulties in comparison with other studies; we had not resorted to such grading histological changes.

There is a histological evidence that the co-administration of methylene blue with rotenone reduces its neuro-toxicity.[288] In our study too, the rodent model pre-treated with captopril, perindopril and losartan had resulted in less severe induction with less motor behavioural changes. Similarly in another study, safranol promoted the differentiation and the survival of dopaminergic neurons in an animal model of PD.[289] With these backgrounds and with our histopathological study outcomes, we

concluded that the drugs acting on the brain angiotensin system had a neuroprotective and a beneficial role in the therapy of PD in toxin induced rodent models.

Immunohistochemistry study in rodent model of PD

Bcl-2 immunohistochemistry is a reliable indication of anti-apoptosis. Bcl-2 is routinely expressed in the limbic cortex, hippocampus, and cerebellar cortex, according to immunohistochemical investigations.[218] The scoring system utilised in this study complied with Tsuyama et al revised criteria for Bcl-2 assessment in blood dyscrasias, particularly B-cell associated leukaemia.[236] Down regulation of Bcl-2 has been reported in 6-OHDA model rat parkinsonism.[290] Studies had quantified the beneficial effects of scorpion venom derived activity peptide[290] and Shudipingchan granules[291] in rat model of parkinsonism in terms of significant increase in Bcl-2 immunohistochemical reactivity.

In the present study, captopril had shown a significant anti-apoptotic property (as evidenced through Bcl-2 immunohistochemistry) in rotenone model. Losartan had also shown a significant anti-apoptotic property (as evidenced through Bcl-2 immunohistochemistry) in MPTP model.

SUMMARY & CONCLUSION

GRAPHICAL ABSTRACT OF THE STUDY

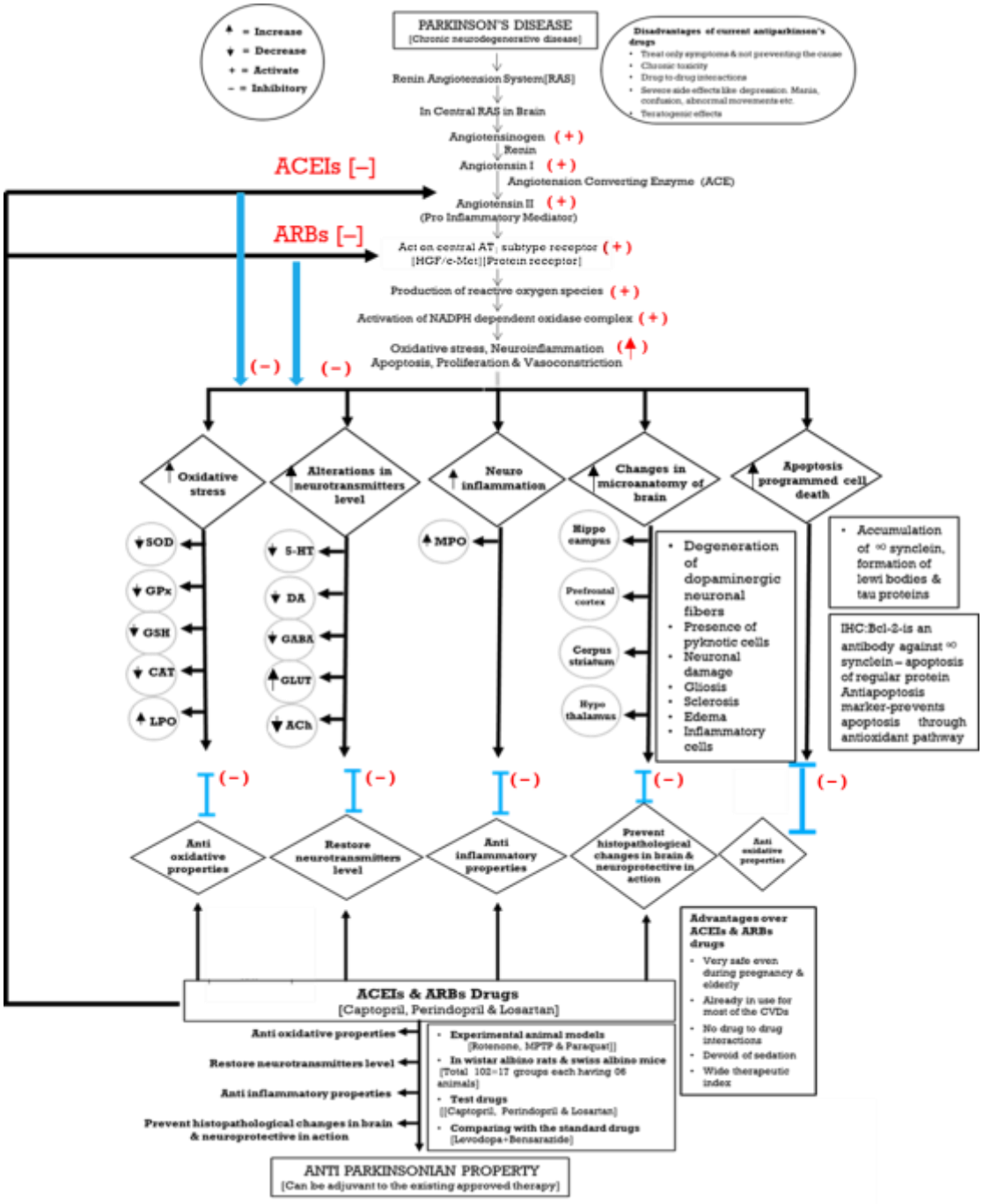

Figure 265: Graphical abstract of the study

SUMMARY

Background and objectives

Drugs used in the parkinsonism therapy centralises on the motor symptoms. Current therapies do not address the disease causation and progression. Issues of neuronal protection and endurance of dopaminergic neurons are seldom addressed in the current therapy. The principal drug, L-dopa has highly limited potential of addressing the non-motor component of PD.

Newer targets for PD treatments have emerged as a result of a better knowledge of the brain renin-angiotensin system and its connection with the hepatocyte growth factor (HGF)/c-Met receptor networks. Vasoconstriction, neuroinflammation, oxidative stress, and apoptosis are all influenced by angiotensin I, II, III, and IV. They do so by engaging on AT1 receptors via angiotensin 1-7 and angiotensin 3-7 subsidiaries. The same angiotensin derivatives that operate on AT2 and AT4 receptors, on the other hand, have been found to cause angiogenesis as well as anti-inflammatory, anti-oxidative, and anti-apoptotic effects. Therefore, this study was designed to evaluate three drugs [captopril, perindopril and losartan] at a time in three rodent models. This was expected to generate a complete and holistic data about the outcomes of effects of these drugs in the rodent models of PD.

The objectives of the present study was to evaluate and compare the anti-parkinson's disease properties among the captopril, perindopril, losartan and with the standard anti-parkinson's disease drug (levodopa) in rotenone induced model in the wistar albino rats, and in MPTP and paraquat induced models in swiss albino mice. The other objective was to evaluate and compare the neuroprotective role of captopril, perindopril and losartan on histoanatomical structures of brain in rotenone, MPTP and

paraquat induced parkinson's disease animal models in wistar albino rats and swiss albino mice.

Methodology:

Healthy adult wistar albino rats of either sex weighing 180-250gm and healthy adult swiss albino mice of either sex weighing 15-20gm were selected. All the animals were obtained from the Central Animal House, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state. Institutional Animal Ethics Committee clearance was obtained before the study. Effects of captopril, perindopril and losartan were studied. Three rodent models namely, rotenone, MPTP, and paraquat models were selected for the study. Parkinsonism was induced accordingly with the injection of rotenone (3 mg/kg BW i.p), MPTP (25 mg/kg BW i.p) and paraquat (25 mg/kg BW i.p) respectively. Each model had six groups with six animals in each group. In each model, First group was normal control group (vehicle control). Second group was negative control with respective toxin induced PD animals. Third group was standard control group induced PD animals received levodopa (12 mg/kg) and benserazide (3 mg/kg BW i.p). Fourth group received captopril (20mg/kg BW i.p). Fifth group received perindopril (5mg/kg BW i.p) and sixth group received losartan (90 mg/kg BW i.p). Motor functions were evaluated by spontaneous locomotor activity, rotarod test and grip strength test. Exploratory behaviour was evaluated by hole board test. Depression influences on the behaviour was studied with forced swim test and tail suspension test. Elevated plus maze test was used for analysing the anxiety influences on behaviour. All the rodents were anaesthetized using thiopental sodium (50 mg/kg) after 24 hours of behavioural monitoring. All the rodents were sacrificed by cervical decapitation. The brain was dissected out of the cranial cavity. Each brain was hemisectioned along the longitudinal fissure into right and left halves. One

hemisection was homogenized and used for the estimation of oxidative stress markers, neurotransmitters and inflammatory marker. Other hemisection was fixed with formalin and used for the histopathological (microanatomical) and immunohistochemistry study. For the evaluation of oxidative stress, superoxide dismutase, reduced glutathione, glutathione peroxidase, catalase and lipid peroxidation were considered. The neurotransmitters - serotonin, dopamine, GABA, glutamate and acetyl choline, and the inflammatory marker myeloperoxidase (MPO) were assayed. Microanatomical (histopathological) evaluation of H & E stained sections of hippocampus, prefrontal cortex, corpus striatum and hypothalamus were carried out and the scoring was given as per the HPE scoring system. Bcl-2 immunohistochemistry was used to evaluate the anti-apoptotic effects of these drugs in the animal models and the scoring system was considered for assessing the immunoreactivity. All continuous data were expressed as mean ± standard deviation. Comparison of the data was done by one way ANOVA. 'P' value of less than 0.05 was taken as significant.

Results

Four percent of the rats/mice that were initially recruited died during the induction of parkinsonism and five percent of the rats/mice died during subsequent evaluation of the properties of angiotensin receptor blockers. Seven percent of the rodents were excluded from the study because of insufficient induction of parkinsonism in various models.

The number of spontaneous activity as measured through actophotometer was significantly more after co-administration of captopril (MPTP and paraquat), perindopril (rotenone and MPTP) and losartan (in all the three models). The mean time spent on the accelerating rotarod is significantly increased in the animals pre-

treated with captopril (rotenone and paraquat model), perindopril (in all the three models) and losartan (in all the three models).

The number of head poking activity was significantly increased with the pre-treatment of captopril (rotenone and paraquat), perindopril (rotenone and paraquat) and losartan (in all the three models). The immobility time in tail suspension test and forced swim test was significantly decreased with pre-treatment of captopril (in all the three models), perindopril (in all the three models) and losartan (in all the three models). The number of entries into the open arm and time spent in the open arm of the elevated plus maze test was significantly increased with the pre-treatment of captopril (in all the three models), perindopril (in all the three models) and losartan (MPTP and paraquat models).

The serotonin and dopamine levels were significantly increased in captopril (in all the three models), perindopril (in all the three models) and losartan (in all the three models). The acetylcholine levels were significantly increased in captopril (in all the three models), perindopril (in all the three models) and losartan (rotenone and paraquat models). The glutamate levels were significantly decreased in perindopril (in all three models) and losartan (in all the three models).

The GSH levels were significantly increased in captopril (in rotenone and MPTP models), perindopril (in rotenone and MPTP models) and losartan (in rotenone and MPTP models). The LPO and MPO levels were significantly decreased in captopril (in all the three models), perindopril (MPTP and paraquat models) and losartan (in all the three models). The SOD and catalase levels were significantly increased in captopril (in all the three models), perindopril (in all the three models) and losartan (in all the three models).

No significant histoanatomical changes were seen in the hippocampus sections; no significant cerebral edema or vacuolations were observed in the corpus striatum and hypothalamus/ thalamus among H&E preparations from the captopril, perindopril and losartan pre-treated animals. Captopril and perindopril had shown 3+ (significant) anti-apoptotic property as evidenced through Bcl-2 immunohistoreactivity in rotenone and paraquat model respectively.

Conclusion:

Captopril, perindopril and losartan had significantly improved the exploratory behavioural aspects in the animal models of PD as evidenced through significant increase in the number of head poking activity of hole board test. Captopril, perindopril and losartan had anti-anxiety effects as evidenced through the significant increase in the number of entries and the time spent in the open arm of elevated plus maze test. Captopril, perindopril and losartan had anti-depression effects as evidenced through the significant decrease in the immobility time in tail suspension test and forced swim test. Captopril, perindopril and losartan significantly decreased the oxidative stress levels in the PD animal model as evidenced through the significant increase in the SOD, oxidized glutathione and Catalase levels, and decrease in the levels of LPO and MPO. Pre-treatment with captopril, perindopril and losartan had shown neuroprotective role (lesser dopaminergic damage, lesser serotonergic damage and lesser GABAergic damage) in the animal models of PD. Captopril, perindopril and losartan had not resulted in the significant histoanatomical changes in the Hippocampus, Prefrontal cortex(cerebrum), Corpus striatum (basal ganglia) and Hypothalamus sections as H&E sections had shown near normal histoanatomy with no significant vacuolations and edema. Captopril and perindopril

had shown a significant anti-apoptotic property as evidenced through Bcl-2 immunohistoreactivity in rotenone and paraquat model respectively.

Overall, captopril, perindopril and losartan had significantly improved the non-motor behavioural aspects of PD. All the three test drugs significantly decreased the oxidative stress levels and were found to be neuroprotective in all the three animal models. Captopril and losartan pre-treated rodents showed a least changes in the histoanatomy of brain in the rodent PD models. Captopril and perindopril were anti-apoptotic in rodent model of PD. Among the three test drugs, losartan had shown a significant anti-parkinson properties in comparison with the standard treatment (levodopa with benserazide).

Limitations and recommendations of the study:

- A study design to note the specific nigrostriatal loss of neurons, either microscopic evaluation or molecular evaluation of apoptotic indicators would have resulted in unambiguous outcomes with regard to neuroprotection.
- All neurotransmitters are estimated in the homogenized hemisections of the brain. Estimation from the specific brain areas like, midbrain, caudal brainstem, would have resulted in a better quantification of effects of the interventional drugs
- Neurotransmitter estimation is a crude way of assessing overall effects. These methods may not delineate the synaptic, presynaptic and dendritic, astrocyte specific concentrations, specific effects and differential outcomes. Quantification of the receptors (by autoradiography) would have resulted in a better understanding of the specific neuro-protective role of these investigational drugs in PD.

- Three models, namely MPTP, rotenone and paraquat models were selected in the study. Even though these models are well established and has been used for beneficial effects of innumerable drugs and molecules in pre-clinical studies in PD, latest 'model fusion' techniques and hemi-parkinsonism induction models are said to be superior to these conventional models in evaluating the beneficial role of these drugs.
- Highly variable motor symptoms and dopaminergic neuronal loss is reported in the previous studies. This necessitates the higher number of animals in each study group. In the present study, such variability between the models were not accounted. Same number of animals were used across all the groups in all the three groups.
- Validity of these models in assessing the symptomatic therapies is questioned recently.
- Mice were used for the paraquat and MPTP models. In the rotenone model, rats were considered. Even though use of rats and mice concurrently has given considerable diversity in the outcome measures, uniform usage of either rats or mice would have resulted in better comparable results.
- In the forced swimming test for the evaluation of antidepressant activity of the rodents, present study concentrated only on the immobility time. Evaluation of climbing efforts and the swimming behaviour would have given deeper understanding of differential effects of serotonin, dopamine and norepinephrine.
- Use of immunohistochemistry to delineate the formation and localization of α-synuclein and Lewy bodies would have significantly added to the outcome of the study

- Sections of midbrain documenting dopaminergic neuronal loss would have quantified effects of the experimental drugs more effectively.

CONCLUSION

☞ ACEIs [Captopril, Perindopril] and ARBs [Losartan] had significantly improved non-motor behavioural aspects in animal model of PD as evidenced through

- Significant increase in the number of head poking activity of hole board test
- Significant decrease in the immobility time in the tail suspension test and forced swim test
- Significant increase in the number of entries and the time spent in the open arm of elevated plus maze test

☞ Captopril, Perindopril and Losartan significantly decreased the oxidative stress enzyme levels in PD animal models as evidenced through

- Significant increase in the SOD, oxidized glutathione and catalase, and decrease in LPO and MPO levels

☞ Captopril, Perindopril and Losartan had shown a neuroprotective role in animal model of PD when pre-treated with these drugs resulted in

- Significantly lesser serotonergic damage (as evidenced through increased serotonin)
- Significantly lesser dopaminergic neuronal loss (as evidenced through increased dopamine)
- Significantly increased acetylcholine and therefore, decreased dopaminergic damage
- Significantly lower glutamate and therefore, decreased dopaminergic damage

☞ Captopril, perindopril and losartan had not resulted in a significant histoanatomical changes in the brain sections as evidenced through

- Near normal architecture of hippocampus
- No significant cerebral edema
- No significant vacuolations in the areas of the brain (hippocampus, cerebral cortex, corpus striatum and hypothalamus)
- No significant cellular inclusion bodies in most areas of the brain (hippocampus, cerebral cortex, corpus striatum and hypothalamus)

☞ Captopril and perindopril had shown a significant anti-apoptotic property as evidenced through

- Bcl-2 immunohistochemistry in rotenone model
- Bcl-2 immunohistochemistry in paraquat model

☞ Among the three test drugs, **Losartan** had shown a significant anti-parkinson properties in comparison with the standard treatment (levodopa with benserazide) as evidenced through

- Significant increase in the immobility time in the tail suspension test (MPTP model)
- Significant increase in the open arm entries and the time spent in the open arm of elevated plus maze test (rotenone model)
- Significant increase in the dopamine (rotenone model), increase in the acetyl choline levels (rotenone and paraquat model), increase in the GABA (MPTP model) and a decrease in the glutamate level (rotenone and MPTP model) indicating a decreased dopaminergic neuronal loss

- Significant increase in the catalase levels indicating effective free radical scavenging (rotenone model)

OVERALL CONCLUSION

☞ ACE inhibitors (captopril & perindopril) possess a significant anti-parkinson properties in rotenone, MPTP & paraquat induced models in wistar albino rats & swiss albino mice.

☞ ARBs (losartan) possess a significant anti-parkinson properties in rotenone, MPTP & paraquat induced models in wistar albino rats & swiss albino mice.

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ANNEXURES

ANNEXURE(s)

MASTER CHART

Behavioural analysis in all the three models												
Sl. No.	Animal models	Group	Actophotometer test	Rotarod test	Grip strength test	Hole board test	Tail suspension test	Force swim test	Elevated plus maze			
									% Open arm preference	No. of entries into the open arm	No. of entries into the close arm	Time spent in the open arm
1	Rotenone [Rat]	I [Vehicle control]	174.33±25.01	118.17±1.83	116.33±3.67	35.50±3.63	26.50±4.30	31.33±3.64	83.33	16.50±1.12	2.67±0.71	168.67±10.73
		II [Negative control]	18.67±4.11	10.17±1.19	8.83±1.80	6.33±1.26	179.17±20.26	165.33±9.12	16.67	3.17±0.48	17.83±1.51	17.50±2.86
		III [Standard control]	161.33±15.25	116.17±2.59	113.17±6.83	31.17±3.46	29.67±4.71	34.50±3.00	83.33	14.67±1.58	3.17±0.87	156.83±11.22
		IV[Captopril]	149.83±17.04	110.17±7.01	109.17±6.88	28.50±2.29	37.17±3.11	41.50±1.91	66.67	11.67±1.05	4.17±0.65	142.83±6.73
		V[Perindopril]	141.83±9.16	108.67±6.49	106.83±8.34	25.33±3.28	41.17±3.28	44.33±2.94	50.00	10.50±1.23	4.50±0.76	140.67±5.52
		VI[Losartan]	154.50±15.42	114.17±5.83	111.50±8.50	29.50±3.70	32.33±2.89	38.33±3.39	66.67	12.67±1.20	3.67±0.49	145.17±9.71
2	MPTP [Mice]	I [Vehicle control]	286.50±8.77	120.00±0.00	120.00±0.00	96.17±5.88	46.50±4.97	51.33±6.08	66.67	25.50±1.48	3.33±0.92	209.50±6.37
		II [Negative control]	21.17±2.70	4.33±1.02	4.33±0.76	4.83±0.95	202.67±7.70	191.17±6.01	16.67	1.83±0.17	30.67±3.53	27.33±2.79
		III [Standard control]	279.17±20.14	119.83±0.17	119.17±0.83	90.50±3.23	50.33±3.81	55.50±4.13	66.67	23.17±1.40	3.83±0.31	206.50±5.40
		IV[Captopril]	267.33±16.01	116.83±2.04	118.67±1.33	84.50±3.87	56.50±2.40	63.83±3.96	66.67	22.17±2.63	5.50±0.67	199.17±8.63
		V[Perindopril]	259.17±17.67	114.83±4.25	117.33±1.76	78.83±6.84	61.67±2.32	70.17±5.49	50.00	19.67±1.89	6.33±0.56	196.67±7.07
		VI[Losartan]	274.67±11.15	118.50±0.96	119.00±0.68	87.83±5.91	53.83±4.00	60.17±6.23	66.67	23.83±2.09	4.17±0.40	202.50±10.17
3	Paraquat [Mice]	I [Vehicle control]	286.50±8.77	120.00±0.00	120.00±0.00	96.17±5.88	46.50±4.97	51.33±6.08	66.67	25.50±1.48	3.33±0.92	209.50±6.37
		II [Negative control]	9.67±1.93	2.50±0.34	2.67±0.33	2.33±0.33	223.17±8.25	206.50±4.61	0.00	1.17±0.17	38.83±3.90	17.67±1.94
		III [Standard control]	281.17±4.69	120.00±0.00	120.00±0.00	95.33±4.23	49.17±2.10	53.83±2.89	66.67	24.67±1.41	3.50±0.22	208.67±4.93
		IV[Captopril]	278.33±9.94	117.83±1.64	119.67±0.33	89.17±4.48	52.17±3.68	56.33±2.67	50.00	20.33±1.94	3.83±0.48	203.50±7.39
		V[Perindopril]	272.83±8.82	117.67±1.38	117.50±1.71	85.83±7.75	55.17±2.69	59.83±6.02	50.00	20.17±2.06	4.17±0.60	199.17±9.49
		VI[Losartan]	280.33±8.69	118.67±0.84	120.00±0.00	91.17±3.41	50.33±2.42	55.17±3.75	66.67	23.33±1.41	3.67±0.33	205.33±8.13

Estimation of antioxidants in all the three models							
Sl. No.	**Animal models**	**Group**	**SOD**	**GPx**	**GSH**	**CAT**	**LPO**
1	**Rotenone [Rat]**	I [Vehicle control]	37.57±2.98	2.72±0.43	35.34±1.72	31.59±0.83	32.48±1.11
		II [Negativecontrol]	78.67±2.22	15.38±1.71	75.97±1.63	72.55±1.67	71.63±1.16
		III [Standard control]	544.48±13.07	43.76±5.13	520.31±15.50	513.69±7.22	508.85±18.61
		IV[Captopril]	7.75±0.41	0.36±0.11	7.14±0.56	5.71±0.90	5.37±0.72
		V[Perindopril]	50.23±4.05	196.36±6.57	61.86±4.00	65.51±2.04	66.04±3.25
		VI[Losartan]	37.57±2.98	2.72±0.43	35.34±1.72	31.59±0.83	32.48±1.11
2	**MPTP [Mice]**	I [Vehicle control]	51.23±1.72	3.98±0.43	49.40±6.07	46.11±1.95	43.62±2.02
		II [Negativecontrol]	87.62±3.09	16.16±2.03	85.46±4.26	81.05±3.56	79.84±6.81
		III [Standard control]	583.65±21.29	34.21±4.81	580.83±6.63	574.79±7.07	570.22±15.63
		IV[Captopril]	6.49±0.76	0.47±0.17	5.74±0.63	4.92±0.71	4.08±0.45
		V[Perindopril]	56.65±2.54	182.85±4.98	59.24±1.60	63.41±3.69	65.98±2.03
		VI[Losartan]	51.23±1.72	3.98±0.43	49.40±6.07	46.11±1.95	43.62±2.02
3	**Paraquat [Mice]**	I [Vehicle control]	51.23±1.72	3.20±0.61	49.10±3.32	45.07±3.78	42.88±4.28
		II [Negativecontrol]	87.62±3.09	4.29±0.65	85.58±2.68	81.34±4.28	79.97±5.37
		III [Standard control]	583.65±21.29	26.59±2.96	578.54±10.76	573.28±2.61	569.80±15.66
		IV[Captopril]	6.49±0.76	0.12±0.02	5.87±0.35	4.93±0.47	4.57±0.37
		V[Perindopril]	56.65±2.54	184.81±6.81	61.43±3.65	65.52±2.31	68.36±4.52
		VI[Losartan]	51.23±1.72	3.20±0.61	49.10±3.32	45.07±3.78	42.88±4.28

Estimation of neurotransmitter & inflammatory marker in all the three models								
Sl No.	**Animal models**	**Group**	**Serotonin**	**Dopamine**	**GABA**	**Glutamate**	**ACh**	**MPO**
1	**Rotenone [Rat]**	I[Vehicle control]	477.28±18.72	54.51±6.16	469.25±21.97	463.12±21.97	458.04±22.75	0.44±0.05
		II [Negative control]	62.37±5.70	4.74±0.69	57.05±2.90	52.43±3.27	50.82±3.51	13.12±1.33
		III [Standard control]	4.95±0.88	0.08±0.02	4.35±0.42	3.95±0.45	3.44±0.39	0.95±0.26
		IV[Captopril]	6.68±0.81	47.86±5.23	9.87±0.71	11.10±0.59	13.04±1.27	1.79±0.29
		V[Perindopril]	38.64±4.24	7.58±1.02	34.63±2.61	32.13±3.20	29.92±2.93	2.29±0.59
		VI[Losartan]	477.28±18.72	54.51±6.16	469.25±21.97	463.12±21.97	458.04±22.75	1.28±0.25
2	**MPTP [Mice]**	I [Vehicle control]	491.84±9.82	51.85±3.97	489.29±22.46	485.51±8.04	483.72±12.39	0.53±0.11
		II [Negative control]	65.50±3.23	7.08±0.69	62.58±6.20	59.15±4.13	54.07±3.23	8.93±0.79
		III [Standard control]	4.46±0.83	0.12±0.03	4.28±0.39	3.97±0.64	3.66±0.52	0.89±0.08
		IV[Captopril]	7.53±0.45	78.27±6.09	8.13±0.94	13.41±1.71	15.09±1.34	1.23±0.10
		V[Perindopril]	36.85±4.03	2.77±0.44	31.21±2.95	29.09±2.36	27.05±2.01	1.51±0.24
		VI[Losartan]	491.84±9.82	51.85±3.97	489.29±22.46	485.51±8.04	483.72±12.39	1.08±0.19
3	**Paraquat [Mice]**	I [Vehicle control]	491.84±9.82	64.83±3.34	486.52±21.26	479.77±13.35	474.83±14.29	0.53±0.11
		II [Negative control]	65.50±3.23	4.06±0.84	59.64±4.30	55.27±4.39	52.38±2.37	10.67±0.95
		III [Standard control]	4.46±0.83	0.07±0.02	4.14±0.25	3.85±0.41	2.86±0.34	1.45±0.29
		IV[Captopril]	7.53±0.45	59.35±3.41	10.37±1.40	12.62±2.07	14.85±1.87	1.81±0.18
		V[Perindopril]	36.85±4.03	6.01±0.95	34.57±1.61	30.66±1.44	29.37±2.02	2.17±0.16
		VI[Losartan]	491.84±9.82	64.83±3.34	486.52±21.26	479.77±13.35	474.83±14.29	1.64±0.39

Histopathological Examination [Score 0-4]						
Sl. No.	**Animal models**	**Group**	**Hippocampus**	**Prefrontal cortex**	**Corpus striatum**	**Hypothalamus**
1	**Rotenone [Rat]**	I [Vehicle control]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		II [Negative control]	4.00±0.17	4.00±0.00	4.00±0.21	4.00±0.00
		III [Standard control]	1.00±0.17	1.00±0.21	0.50±0.22	1.00±0.31
		IV [Captopril]	1.50±0.22	2.00±0.21	1.00±0.21	2.00±0.21
		V [Perindopril]	1.50±0.40	2.00±0.31	2.00±0.37	2.00±0.17
		VI [Losartan]	1.00±0.26	1.00±0.17	0.50±0.33	1.00±0.17
2	**MPTP [Mice]**	I [Vehicle control]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		II [Negative control]	4.00±0.21	4.00±0.17	4.00±0.00	4.00±0.00
		III [Standard control]	1.00±0.17	1.00±0.17	1.00±0.21	1.00±0.17
		IV [Captopril]	1.00±0.21	2.00±0.21	2.00±0.17	1.50±0.3
		V [Perindopril]	2.00±0.00	2.00±0.31	2.00±0.21	2.00±0.31
		VI [Losartan]	1.00±0.17	1.00±0.17	1.50±0.22	1.00±0.17
3	**Paraquat [Mice]**	I [Vehicle control]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		II [Negative control]	4.00±0.21	4.00±0.17	4.00±0.17	4.00±0.00
		III [Standard control]	1.00±0.21	1.00±0.17	1.00±0.17	1.00±0.26
		IV [Captopril]	2.00±0.37	1.50±0.22	2.00±0.31	1.00±0.21
		V [Perindopril]	2.00±0.31	2.00±0.37	2.00±0.31	2.00±0.26
		VI [Losartan]	1.00±0.21	1.00±0.17	1.00±0.17	1.00±0.17

****Scores 0**= Histological section undistinguishable from control group [Number of healthy neurons appeared normal, even if few pyknotic cells found], **1**= More than 75% of healthy pyramidal cells with others 25% with clear evidence of cell death, **2**= 50-74% of healthy pyramidal cells, **3**= 25-49% of healthy pyramidal cells and **4**= Less than 25% of healthy pyramidal cells.

Immunohistochemistry Examination [Score 0-5]						
Sl. No.	**Animal models**	**Group**	**Hippocampus**	**Prefrontal cortex**	**Corpus striatum**	**Hypothalamus**
1	**Rotenone [Rat]**	I [Vehicle control]	5.00±0.17	5.00±0.00	5.00±0.00	5.00±0.00
		II [Negative control]	0.00±0.17	0.00±0.21	0.00±0.17	0.00±0.00
		III [Standard control]	4.00±0.37	4.00±0.17	4.00±0.26	5.00±0.34
		IV [Captopril]	3.50±0.22	3.50±0.33	3.00±0.48	3.50±0.33
		V [Perindopril]	3.50±0.49	3.00±0.21	3.00±0.48	2.50±0.22
		VI [Losartan]	4.00±0.26	4.00±0.37	4.00±0.31	4.00±0.21
2	**MPTP [Mice]**	I [Vehicle control]	5.00±0.00	5.00±0.00	5.00±0.21	5.00±0.17
		II [Negative control]	0.00±0.00	0.00±0.17	0.50±0.22	1.00±0.21
		III [Standard control]	4.00±0.17	4.00±0.17	4.00±0.21	4.50±0.40
		IV [Captopril]	3.50±0.33	4.00±0.21	3.50±0.33	4.00±0.34
		V [Perindopril]	3.00±0.31	3.50±0.22	3.00±0.21	3.00±0.37
		VI [Losartan]	4.00±0.17	4.00±0.26	4.00±0.31	4.00±0.37
3	**Paraquat [Mice]**	I [Vehicle control]	5.00±0.21	5.00±0.00	5.00±0.17	5.00±0.00
		II [Negative control]	0.00±0.17	0.00±0.17	0.00±0.21	0.00±0.17
		III [Standard control]	4.00±0.21	4.00±0.31	4.00±0.17	4.00±0.31
		IV [Captopril]	2.50±0.40	3.50±0.22	4.00±0.17	4.00±0.34
		V [Perindopril]	2.50±0.33	3.50±0.33	3.00±0.17	3.00±0.21
		VI [Losartan]	4.00±0.34	4.00±0.26	4.00±0.26	4.00±0.26

***Scores:** [**0:** Nil/No neuroprotection, **1:** Plus^{+} (Mild Neuroprotection), **2:** Plus^{++} (Borderline Neuroprotection), **3:** Plus^{+++} (Good Neuroprotection), **4:** Plus^{++++} (Excellent/Normal Neuroprotection) and **5:** >Plus^{++++} (Excellent/Normal Neuroprotection)]

Shri B. M. Patil Medical College, Hospital and Research Centre
B.L.D.E UNIVERSITY, BIJAPUR, Karnataka-586103

Department of Anatomy

Proforma for Collection of Sample

Title of the Study: Evaluation of Neuroprotective Role of Drugs that Modify Renin Angiotensin System on Histoanatomical Structures of Brain in Experimentally Induced Animal Models of Parkinson's Disease

Date: ……. Strain: ……. Animal Model: …….

Group No.: ……. Group Description: …….

Sex of Animal: ……. Weight of Animal [Grams]: …….

Test Drug: ……. Experimental Drug: …….

Dose of Test Drug: ……. Dose of Experimental Drug: …….

Humidity: ……. Lab. Temperature: …….

Sl. No.	Behavioral Tests [Rotenone, MPTP & Paraquat Models]							
	Animal No.		1	2	3	4	5	6
I.	**Motor Functions**							
	A.	Locomotor activity using actophotometer; [Total number of counts in 10 minutes						
	B.	Motor co-ordination using rota rod apparatus[Fall of time in seconds]						
	C.	Grip strength using grip strength test; [Fall of time in seconds]						
II.	**Exploratory Behaviour**							
	A.	Exploratory behaviour by using hole board test[Number of nose pickings in 5 minutes]						
III.	**Depression Behaviour**							
	A	Tail Suspension Test [TST]; Immobility time in seconds [in total 5 minutes test]						
	B	Forced Swim Test [FST]; Immobility time in seconds in last 4 minutes [of total 6 minutes test]						
IV.	**Anxiety Behaviour By Using Elevated Plus Maze**							
	A.	First arm [Open/Closed] preference						
	B.	Number of entries into the open arm						
	C	Number of entries into the closed arm						
	D	Time [in seconds] spent in open arm						

Biochemical assay and Microanatomical changes in different parts of brain of rodents for all the three models

	Total Protein	Anti-oxidants					Neurotransmitters					Infl. marker	HPE**				IHC***			
		SOD	GPx	GSH	CAT	LPO	5-HT	GABA	GLU	ACh	LPO	MPO	H	PC	CS	Hypo	H	PC	CS	Hypo
1																				
2																				
3																				
4																				
5																				
6																				

****Scores 0**= Histological section undistinguishable from control group [Number of healthy neurons appeared normal, even if few pyknotic cells found], **1**= More than 75% of healthy pyramidal cells with others 25% with clear evidence of cell death, **2**= 50-74% of healthy pyramidal cells, **3**= 25-49% of healthy pyramidal cells and **4**= Less than 25% of healthy pyramidal cells.

*****Scores:** [**0:** Nil/No neuroprotection, **1:** Plus^{+} (Mild Neuroprotection), **2:** Plus^{++} (Borderline Neuroprotection), **3:** Plus^{+++} (Good Neuroprotection), **4:** Plus^{++++} (Excellent/Normal Neuroprotection) and **5:** >Plus^{++++} (Excellent/Normal Neuroprotection)]

Signature of Guide/Co-Guide **Signature of Principal Investigator**

CONFERENCE PRESENTATIONS

Oral/Poster Presentations

PR/OR/14

Angiotensin Converting Enzyme inhibitors exhibited favourable effects on histoanatomical structures of brain in rotenone induced animal models of Parkinson's disease: a preclinical study

Prakash KG, Bannur BM, Madhavrao C, Saniya K and Mythili Bai K

Presented at **12th Annual International Conference (SAC-ACCP)**, Clinical Pharmacology: Preparing for the future organized by **South Asian College of Clinical Pharmacology**, An Affiliate of **American College of Clinical Pharmacology**in association with Indian Council of Medical Research (ICMR), Maharashtra University of Health Sciences and Department of Pharmacology, Seth GS Medical College and KEM Hospital Mumbai conducted at Mumbai Cricket Association Recreation Centre, Kurla, Mumbai on 17th and 18th April 2019

PHA034

Neuroprotective role of angiotensin converting enzyme inhibitors on histoanatomical structures of brain in MPTP induced animal models of Parkinson's disease: a pre-clinical study

Prakash KG, Bannur BM, Madhavrao C, Saniya K and Mythili Bai K

Presented at **MED Inspire**, an international multidisciplinary medical summit, organized by **Dr. D Y Patil University,**at **Dr. D Y Patil University Capmus, Navi Mumbai** on 14th to 16th February 2019.

RESEARCH PUBLICATIONS

Prakash K. G, Bannur B. M, Madhavrao C, Saniya K, Neurobehavioral Effects of Losartan on RotenoneInduced Parkinsonism in Rats. Indian Journal of Public Health Research & Development 2019; 10 (9): 38-43

Available from:

https://www.indianjournals.com/ijor.aspx?target=ijor:ijphrd&volume=10&issue=9&article=008

Prakash K. G, Bannur B. M, Madhavrao C, Saniya K, Viveka S, Sudha M J. Neurobehavioral and neuroprotective role of captopril in the rotenone model of rat Parkinsonism. Int J Res Pharm Sci 2019; 10(4): 3523-53.

Available from: https://pharmascope.org/index.php/ijrps/article/view/1728/2397

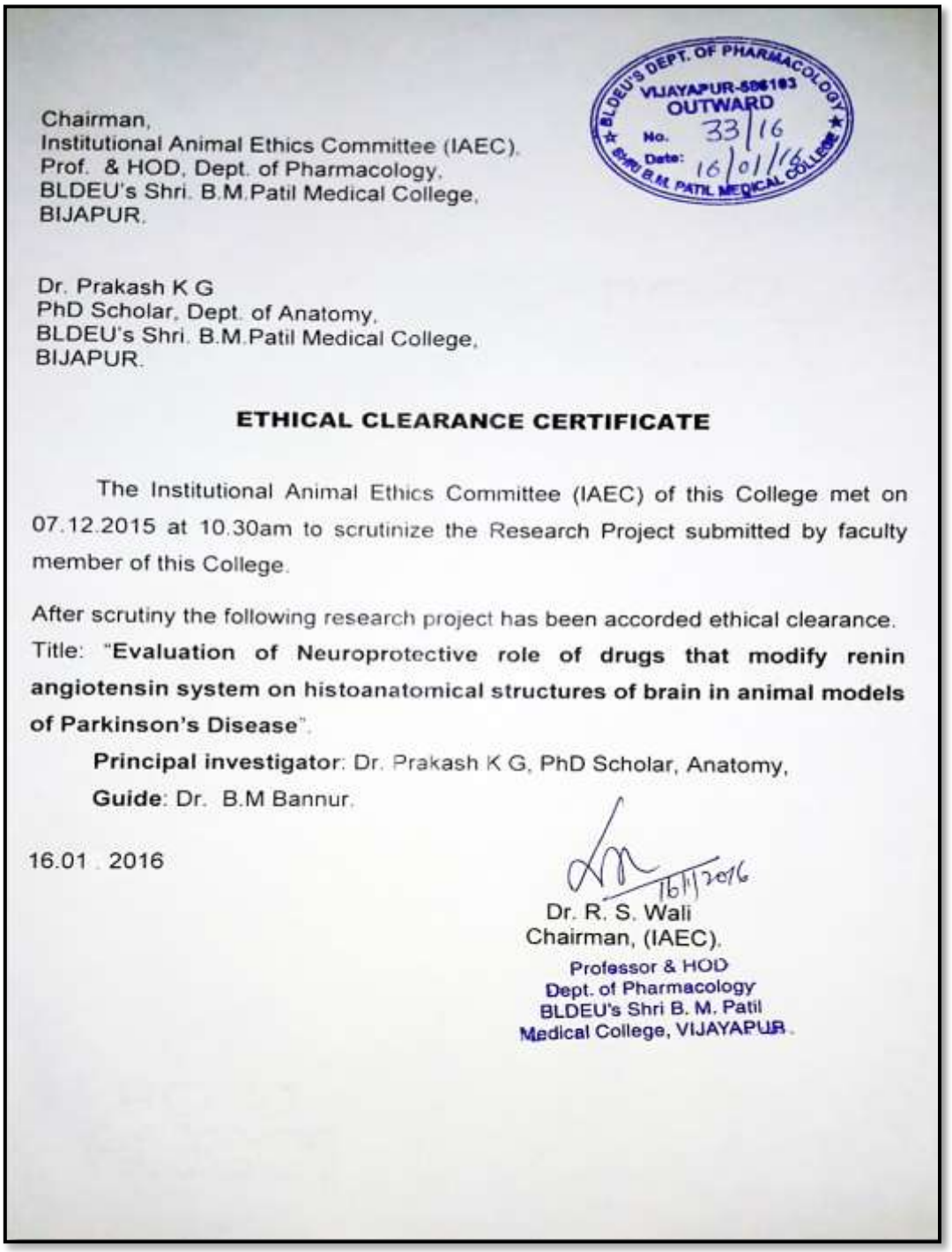
Prakash K G, Bannur B M, Chavan MD, Saniya K, Sailesh KS, Rajagopalan A. Neuroanatomical changes in Parkinson's disease in relation to cognition: An update. J Adv Pharm Technol Res 2016;7:123-6

Available from: http://www.japtr.org/text.asp?2016/7/4/123/191416

Prakash K. G, Bannur B. M, Madhavrao C, Saniya K, Viveka S, Sudha M J. Anti-Depressant and Neuroprotective Effects of Captopril and Perindopril in Paraquat Model of Parkinsonism. Biomed Pharmacol J 2019;12(4).

Available from: https://biomedpharmajournal.org/vol12no4/anti-depressant-and-neuroprotective-effects-of-captopril-and-perindopril-in-paraquat-model-of-parkinsonism/

Institutional Ethical Clearance Certificate



BLDEU'S DEPT. OF PHARMACOLOGY VIJAYAPUR-586103 OUTWARD No. 33/16 Date: 16/01/16 SHRI B.M. PATIL MEDICAL COLLEGE

Chairman,
Institutional Animal Ethics Committee (IAEC).
Prof. & HOD, Dept. of Pharmacology,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

Dr. Prakash K G
PhD Scholar, Dept. of Anatomy,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 07.12.2015 at 10.30am to scrutinize the Research Project submitted by faculty member of this College.

After scrutiny the following research project has been accorded ethical clearance.

Title: "**Evaluation of Neuroprotective role of drugs that modify renin angiotensin system on histoanatomical structures of brain in animal models of Parkinson's Disease**".

Principal investigator: Dr. Prakash K G, PhD Scholar, Anatomy,

Guide: Dr. B.M Bannur.

16.01 . 2016

16/1/2016

Dr. R. S. Wali
Chairman, (IAEC).
Professor & HOD
Dept. of Pharmacology
BLDEU's Shri B. M. Patil
Medical College, VIJAYAPUR.

BLDE (DEEMED TO BE UNIVERSITY)

PLAGIARISM VERIFICATION CERTIFICATE

1. **Name of the Student:** Dr Prakash K G **Reg No:** 14PHD007

2. **Title of the Thesis:** Evaluation of Neuroprotective Role of Drugs that Modify Renin Angiotensin System on Histoanatomical Structures of Brain in Animal Models of Parkinson's Disease

3. **Department:** Anatomy

4. **Name of the Guide & Designation:** Dr. B. M Bannur, Professor

5. **Name of the Co Guide & Designation:** Dr. Madhavrao C, Professor

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