NEUROPROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN AGAINST MPTP INDUCED NEURODEGENERATION IN RATS.

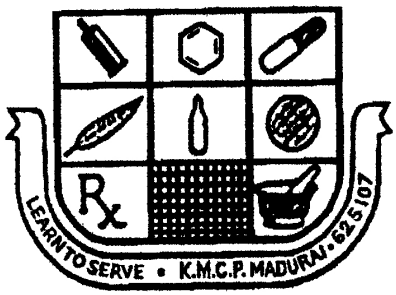
Dissertation submitted in partial fulfillment of the Requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI



DEPARTMENT OF PHARMACOLOGY

K.M.COLLEGE OF PHARMACY

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APRIL-2015

ACRONYMS

1. CNS : Central Nervous System
2. WHO : World Health Organisation
3. ATP : Adenosine triphosphate
4. PL : Phospholipase
5. PLA_2 : Phospholipase A_2
6. Hr : hour
7. i.p : intraperitoneally
8. p.o : orally
9. PKC : Phosphokinase C
10. PLC : Phospholipase C
11. DAG : Diacylglycerol
12. mGluR : Metabotopic Glutamate Receptors
13. PIP_2 : Phosphatidyl inositol diphosphate
14. Ca^{2+} : Calcium
15. IP_3 : Inositol triphosphate
16. O_2^- : Superoxide radical
17. H_2O_2 : Hydrogen peroxide
18. OH^- : hydroxyl ion
19. NAD^+ : Nicotinamide adenine dinucleotide
20. NADH : Nicotinamide adenine dinucleotide reduced
21. $NF_\kappa B$: Nuclear factor kappa B
22. γGTT : γ Glutamyl transpeptidase
23. VSCC : Vesicular Storage Calcium Channel

24. ROS-Reactive oxygen species

25. NO : Nitric oxide

26. PD : Parkinson’s disease

27. MPTP : 1,2,3,6 methyl phenyl tetrahydropyridine

28. MAO-B : Monooxidase type B

29. MPP+ : Methyl pyridinium ion

30. UCHL1 : Ubiquitin C-Hydroxylase 1

31. SN : Substantia nigra

32. DA : Dopamine

33. DOPAC : 3-phenyl dihydroxy phenyl acetic acid

34. MAO : Monoamine oxidase

35. Mn –SOD : Manganese Superoxide dismutase

36. NMDA : N-methyl D-Asparatate

37. NAD : Nicotinamide dinucleotide

38. AIF : Apoptosis inducing factor

39. PARP : Poly (ADP-ribose) polymerase

40. iNOs : Inducible nitric oxide synthase

41. SNpc : Substantia nigra pars compacta

42. MDA : Malonidialdehyde

43. TNF : Tumour Necrotic factor

44. GSH : Glutathione

45. RAE : Rhodiola aqueous extract

46. UPS : Ubiquitin proteosome system

47. GDNF : Glial Derived Neurotropic factor

48. 6-OHDA : ortho hydroxy dopamine

49. DAT : Dopamine transporter

50. IL : Interleukin

51. nNos : Neural nitric oxide synthase

52. GPx : Glutathione peroxidase

53. HT : Hydroxytryptamine

54. Bnz:Benzamide

55. NI:Nitroindazole

56. ZNS : Zonisamide

57. Fe^{2+} : Ferrous ion

58. Fe^{3+} : Ferric ion

59. NH_3 : Ammonia

60. NE : Norepinephrine

61. HAMD : Hamilton Rating Scale for Depressive scores

62. BDI : Beck Depression inventory scores

63. GR : Glutathione reductase

64. CMC : Carboxymethyl cellulose

65. IFN : Interferon

66. AchE : Acetylcholine Esterase

67. APAP : Acataminophen

68. CCl_4 : Carbon tetrachloride

69. BrdU : Bromodeoxyuridine

70. HVA : Homovanillic acid

71. Eg : Example

72. LPO : Lipid hydroperoxides

73. XO : Xanthine oxidase

74. PCC : Protein carbonyl content

75. BD :Boerhaavia diffusa

76. BDE: Boerhaavia diffusa Extract

77. ITL: Initial transfer latency

78. RTL : Retention transfer latency

79. HA : Hyaluronic acid

80. PCIII : Pro-collagen III

81. CIV : Collagen IV

82. PBMCs : Peripheral Blood mononucleocytes

83. TA : Total antioxidants

84. LBs : Lewy bodies

85. SPECT : Single Photon Emission Tomography

86. ELISA : Enzyme Immunosorbent assay

87. GABA : Gamma aminobutryic acid

88. GSSG : Reduced Glutathione

89. CAT : Catalase

CERTIFICATE

This is to certify that the dissertation entitled **"NEUROPROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN AGAINST MPTP INDUCED NEURODEGENARTION IN RATS",** is a bonafide work done by **Mr. NIRUBAN CHAKKARAVARTHI.G , Reg.No:261325056** at K.M.College of pharmacy, Uthangudi**,** Madurai – 107, in partial fulfillment of the university rules and regulations for the award of **Master of Pharmacy in Pharmacology** under my guidance and supervision during the academic year of 2013 – 2014. This dissertation partially or fully has not been submitted for any other degree or diploma of this university.

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ACKNOWLEDGEMENT

"The dream begins with a teacher who believes in you, who tugs and pushes you to the next plateau, some times poking you with a sharp stick called knowledge."

Its affords me an immense pleasure to acknowledge with gratitude the help, guidance and encouragement rendered to me by all those people to whom I owe a great deal for the successful completion of this endeavour.

At this venue I take this opportunity to acknowledge all those who have helped me a lot in bringing the dissertation work. Without their input this undertaking would have not been complete.

With deep sense of gratitude and veneration I express my profound sense of appreciation and love to my parents **Mr.Gunalan.S and Mrs.Pushpa.G**, and to my uncle **Mr.P.Kalidoss B.sc, M.B.A, CPI** for providing me love like caring and support for all my effort .I can never thank enough them for sacrificing their present for my future.

I am greatful to thank our most respected correspondent **Prof. M. Nagarajan.,M.Pharm.,M.B.A.,DMS(BM)**, K.M.College of Pharmacy, Madurai, for providing necessary facilities to carry out this thesis work successfully.

It's my previleage to express my heartful gratitude to our beloved Principal; **Dr. S. Venkataraman.,Ph.D.,**Principal & Head of the Department of Pharmaceutical Chemistry, K. M. College of Pharmacy,Madurai, for his all inspiration in bringing out this work a successful one.

I wish to express my sincere gratitude to my respected Vice Principal; **Dr.N.Chidambaranathan.,M.Pharm.,Ph.D.**, Professor &Head of the Department of Pharmacology K. M. College of Pharmacy, Madurai, for his immense guidance, help, dedicated support, intelleuctual supervision and professional expertise he has best owed upon me for the timely completion of this work. I thank him for the freedom of thought, trust, and expression which he best owed on me.

Its gives me immense pleasure in extending my heartfelt thanks to my respected guide, **Mrs. G.Nalini, M.Pharm., (Ph.D).,** assistant professor Department of Pharmacology, K.M. College of Pharmacy, Madurai, for being a well wisher and an interested person in seeing my performance. Due to her selfless efforts, help, guidance and encouragement in all stages of my work help in completion of this thesis work.

"Thank You mam " for all you done for me

It is pleasure to give express my thanks to my pharmacology department teaching staff **Mr. N. Jegan, M.Pharm., Mr.M.Santhanakumar,M.Pharm., Mr.Marimuthu,M.pharm** for helping me for completion of this work.

I also extended my gratitude to **Dr.D.Stephan.,** The American College Lecturer, Department of Botany, Madurai. for providing me with the plant specimen for my project work.

Thanks to our lab technician **Mrs.S.Revathi D.Pharm** and our lab attender **Mrs.C. Nallammal** for helping me taking care of my experimental animals.

I will always be thankful to our librarian **Mrs.M.Shanthi B.A.**,**M.Phil** library assistant **Mrs.Angelo Merina Priya**, and all other **teaching** and **non teaching staffs** of our college.

I also extend my gratitude to Management and Staffs of **Apollo Lab, Madurai** for conducting haematological and histopathological studies.

I am very much indebted to my beloved brothers **Mr.A.S.Loganathan M.Pharm ., Mr.Abbas B.Pharm** who is living in the depth of my heart,

With a deep sense of love, I express endless thanks to my B.pharm Batch mates **Mr. Abdulhalik , Mr.Praveenkumar , Mr.Rameshbabu , Mr.Thiruppathi , Mr.Selvam , Mr. Pandiselvam ,** and for their support throughout my courses.

with deep sense of affection I express my endless gratitude to my close friends & My M.Pharm My seniors **Miss. Asha Ajayan M.Pharm.,** My classmates **Mr.Manikandan., Mr.Yohesh Prabhu Mrs.Sanitha.,** & My juniors **Miss.suba** & **Miss.annapoorni.**

Special thanks to Pharmapredators ... Pharmawarrious,.

CONTENTS

INTRODUCTION

Neuropharmacology is one of the branches of Pharmacology that encompasses many aspects of the nervous system from single neuron manipulation to entire areas of the brain, spinal cord and peripheral nerves. It deals with the study of how drugs affect cellular function in the nervous system.[1,2] It brings to understand how human behaviour and thought process are transferred from neuron to neuron and how medications can alter the chemical foundation of these processes.

Two main branches of Neuropharmacology:

1. Behavioural Neuropharmacology
2. Molecular Neuropharmacology

Behavioural Neuropharmcology:

It focuses on the study of how drugs affect human behaviour including the study of how drug dependence and addiction affect human behaviour.[3]

Molecular Neuropharmacology:

It focuses on the study of neurons and their neurochemical interactions.

Both fields are interconnected. These are concerned with the interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators, enzymes, second messengers, Co-transporters, ion channels and receptor protein in the central and peripheral nervous system.

With the help of neurochemical interactions researchers are developing drugs to treat many different neurological disorders including pain, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease and Psychological disorders such as addiction.

BASIC PRINCIPLES OF THE NEUROPHARMACOLOGY

Neurological diseases affect a large fraction of the general population.The pathophysiological mechanisms underlying most brain disorders are poorly understood. Many CNS disorders have a genetic basis.The elucidation of mutations in familial forms of these diseases contribute to our understanding of their pathophysiology.[(4)]

Brain diseases are classified as follows:

Psychiatric diseases

- Neurodevelopment disorders (Autism, Rett syndrome, Attention deficit disorders)
- Anxiety (Panic, Generalized anxiety, Phobia, Post traumatic stress disorder)
- Mood disorders (Depression, Bipolar disorder)
- Schizophrenia, Tourette's Disease
- Drug dependence

Neurological diseases

- Stroke and Ischemia
- Brain lesions (Trauma, Tumors, Infections)
- Epilepsy
- Chronic pain
- Sleep disorders
- Movement disorders (Dystonia, Tremors)

Autoimmune diseases

- Multiple Sclerosis
- Myaesthenia gravis

Neurodegenerative diseases

- Alzheimer's disease
- Parkinson's disease
- Huntington's disease
- Amyotropic lateral sclerosis
- Prion disease (Crutzfeld Jacob disease)

NEURODEGENERATIVE DISEASES

The term 'Neurodegenation' means progressive loss of structure or function of neurons. Neurodegenerative diseases are group of illness with distinct clinical phenotypes and genetic etiologies characterized by progressive and irreversible loss of neurons from specific regions of the brain.[5]Parkinson's disease, Alzheimer's and Huntington's disease occurs as a result of neurodegeneration. WHO data suggest that neurological and psychiatric disorders are important and growing cause of morbidity. The magnitude and burden of mental, neurological and behavioural disorders is huge, affecting more than 450 million people globally. According to the Global Burden of Disease report, 33 percentage of years lived with disability and 13 percent of disability-adjusted life years are due to neurological and psychiatric disorders, which account for four out of the six leading cause of years lived with disability.[6] Neurodegenerative disorders such as Alzheimer's and Parkinson's disease account for a significant and increasing proportion of morbidity and mortality in the developed world.As a result of increased life expectancy and changing population demographics, neurodegenerative dementias and neurodegenerative movement disorders are becoming more common.[7,8]

The most important factors related to neurodegeneration are oxidative stress, excitotoxicity, energy metabolism and ageing, environmental triggers and genetics. Oxidative stress and excitotoxicity are two important targets for neuroprotective therapy.

Oxidative stress:

It is caused by excessive production of reactive oxygen species. The brain utilized mitochondrial oxidative phosphorylation for generating ATP, the key molecule of energy. Under certain conditions highly reactive oxygen species may be generated as side products of this process. ROS attack many key molecules such as superoxide dismutase, catalases as well as antioxidants involved in antioxidant defense mechanisms.

Excitotoxicity:

The phenomenon of Glutamate accumulation in the neurons is called excitotoxicity. Calcium overload is the essential factor in this process, which leads to cell death. It causes neurotoxicity by increased release of glutamate, activation of proteases and lipases, which disrupt mitochondrial membrane and activation of endothelium leads to activation of nitric oxide synthase inturn, produce NO. Its high concentration leads to produce free radicals.[9]

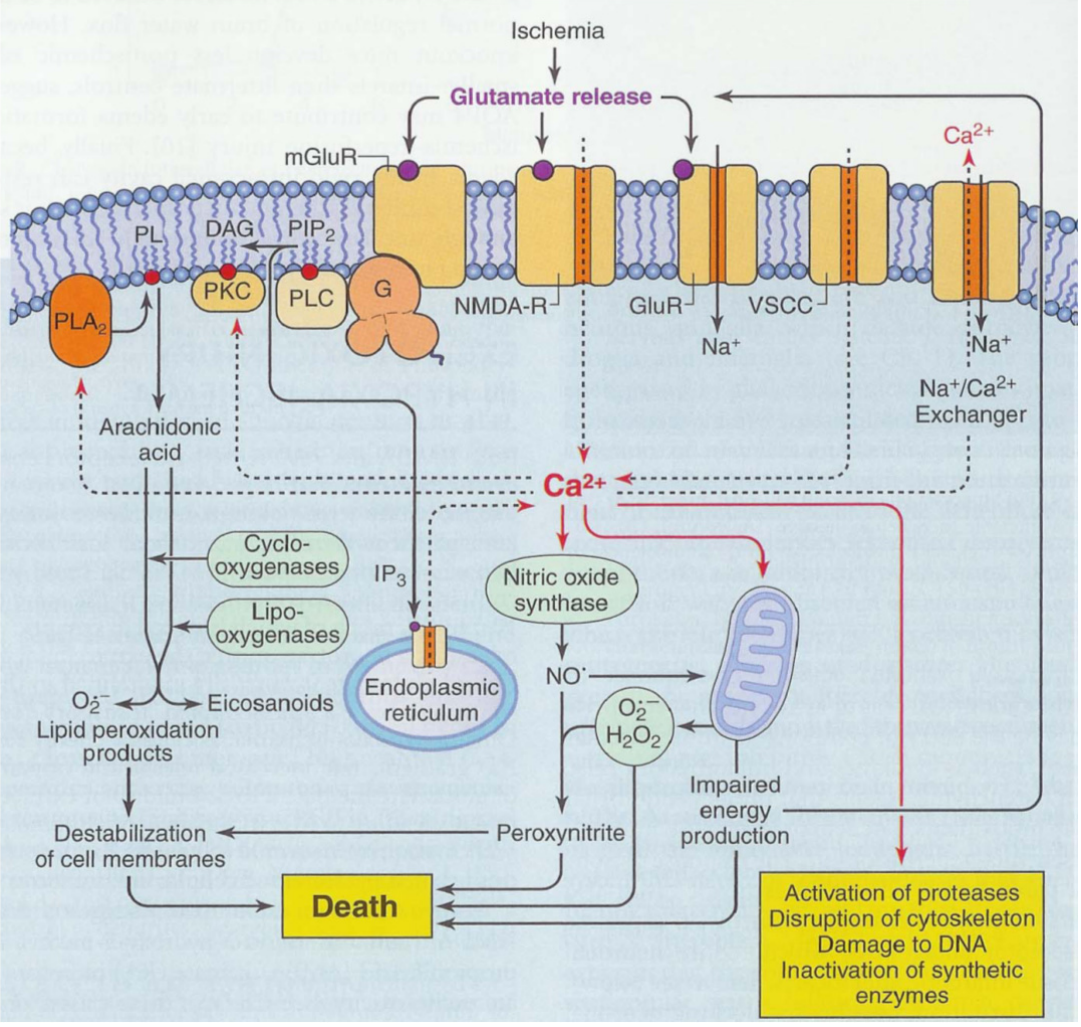


FIG. NO: 1

The important characteristic of neurodegenerative disorder is that particular anatomic or physiologic system of neurons is selectively affected. Degenerative diseases are classified into individual syndromes based on clinical aspects and anatomical distribution of lesions.[10]

Table no.1:-COMMON NEURODEGENERATIVE DISORDERS.

REGION AFFECTED	DISEASE	MAIN FEATURES	PREDOMINANT PATHOLOGY
Cerebral cortex	Alzheimer's disease	Progressive senile dementia.	Cortical atrophy, senile plaques (neuritis), neurofibrillary tangles, amyloid angiopathy.
	Pick's disease	Pre-senile dementia.	Lobar cortical atrophy, ballooning degeneration of neurons.
Basal ganglia and Brain stem	Huntington's disease	Progressive dementia with choreiform movements.	Atrophy of frontal lobes fibrillary astrocytosis.
	Parkinson's disease	Abnormalities of posture movements.	Aggregates of melanin containing nerve cells in brain stem, intracytoplasmic neuronal inclusions (Lewy bodies).
Spinal cord and cerebellum	Cerebellar cortical degeneration	Progressive cerebellar ataxia.	Loss of purkinjee cells in cerebral cortex.
	Olivopontocerebellar Atrophy	Cerebellar ataxia.	Combination of atrophy of cerebellar cortex, inferior olivary nuclei and pontine nuclei.
	Spinocerebellar atrophy	Gait ataxia. Dysaetheia.	Degeneration of spinocerebellar tracts, peripheral axon myelin sheaths.
Motor neurons	Amyotropic lateral sclerosis	Syndromes of muscular weakness and wasting without sensory loss.	Progressive loss of motor neurons both in cerebellar cortex and in the anterior horn of spinal cord.
	Werdning-Hoff man disease	Spinal muscular atrophy in infants.	Loss of motor neurons, denervation, atrophy of muscles.

PARKINSON'S DISEASE

Parkinson's disease is a common and debilitating age-associated human neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta and degeneration of projecting nerve fibres in the striatum which leads to extrapyramidal motor dysfunction.[11]It was first documented by James Parkinson and it called so in 1817 as the "Shaking Palsy"an essay written by him.

EPIDEMIOLOGY:

Parkinson's disease is the second most common age-related neurodegenerative disorder. It develops much less frequently than Alzheimer's disease ranging from 0.1%-5% annually.[12] PD increases with age in both men and women but the rate in men exceeds that women by two-fold.[13] Worldwide estimates vary 15/100,000 in China, 657/100,000 in Argentina, 100-250/100,000 in North America and Europe. PD is more common in white people in Europe and North America and lower rates in China, Nigeria and Sardinia.

Its prevalence is 1% among population over 65years and 2% over 80years.The annual incidence rates for PD ranges from 110-330/100,000 individuals over age 50[14] and after age 80years the incidence rate increases to 400-500 individuals/100,000 annually. Among persons over age 65 the prevalence of Parkinson's disease has been estimated at 1800 per 100,000 (1.8%) individuals, increasing from 600 per 100,000(0.6%) for persons between the age of 65 and 69 to 2600 per 100,000 (2.6%) for those 85 to 89 years.[15] 600, 000 to 1 million individuals in the United States have Parkinson's disease, and approximately 70, 000 develop the disease each year. Risk factors related to PD are ageing, head trauma and declining oestrogen levels.

ETIOLOGY:

The specific etiology of Parkinson's disease is not known. Epidemiological studies indicate that a number of factors may increase the risk of developing Parkinson's disease. Both genetic and environmental factors have been implicated as a cause of Parkinson's disease.[16]

Environmental factors:

A number of exogenous toxins have been associated with the development of Parkinson's disease such as pesticides, herbicides, trace metals, cyanide, and lacquer thinner, organic solvents, carbon monoxide and carbon disulphide.

The most important toxin related to the pathogenesis of Parkinson's disease is 1, 2, 3, 6-methyl phenyl tetrahydropyridine (MPTP). It is a byproduct of illicit manufacture of synthetic meperidine derivative. MPTP induces toxicity by [17,18]

- Its conversion in astrocytes to the pyridinium ion (**MPP^+**) in a reaction catalysed by mono oxidase type - B (**MAO-B**).
- MPP^+ is then taken up by dopamine neurons and causes a mitochondrial complex-I defect similar to that of Parkinson's disease.

Genetic factors:

Genetic factors play an important role in the pathogenesis of Parkinson's disease.Genes responsible for familial Parkinsonism is α-synuclein, parkin, UCHL1 and DJ1.[19]

α-synuclein is a small flexible monomeric protein of 140 aminoacids.It is abundantly expressed in the nervous system in which it is concentrated in pre-synaptic terminals. It is widely expressed in various brain regions[20] including neocortex, hippocampus, dentate gyrus, olfactory bulb, thalamus and cerebellum and also in the amygdala and nucleus accumbens.Its normal function is unknown but it may have a role in synaptic vesicle transport and preserving synaptic plasticity.[21]

Mutation in the α-synuclein causes fibrillogenesis, leading to increased self aggregation of protein and finally forms lewy bodies.[22]

Parkin is a protein encoded by PARK2 gene. It is a part of the ubiquitin-proteosome system that mediates the targeting of proteins for degradation.[23]

Mutations in parkin could result in the accumulation of misfolded substrate proteins in the endoplasmic reticulum, resulting in cell death.

The important factors related to **pathogenesis of Parkinson's disease** are

- Ageing
- Oxidative stress
- Glutathione depletion
- Nutritional deficiency
- Metals such as Iron

Ageing:

- The risk of Parkinson's disease is clearly age dependent.
- As age increases loss of striatal dopamine and loss of dopamine cells in substantia nigra occurs.[24]
- Due to increase in age the antioxidant defense system get impaired, which fail to scavenge free radicals produced during oxidative phosphorylation ,attack mitochondrial membrane which further leads to cell death.

Oxidative stress:

Oxidative stress contributes to the cascade leading to dopamine cell degeneration in Parkinson's disease. Oxidative stress hypothesis refers a imbalance between formation of hydrogen peroxide and oxygen derived free radicals such as hydroxyl ion (OH^-**)** and superoxide radicals (O_2^-) can cause cell damage due to chain reaction of membrane lipid peroxidation.[25]In brain substantia nigra is more vulnerable to oxidative stress than other regions. Its unique features are as follows

- It contains high content of dopamine which consequent to the high density of dopaminergic neurons. Dopamine has a strong tendency to spontaneously breakdown into oxidant metabolites by autooxidation most reactive among these autometabolites are 6-hydroxydopamine quinone and dopamine aminochrome.[26] Dopamine's oxidative breakdown can be accelerated by free iron or by other redox active elements such as copper, zinc or manganese.[27]
- High content of iron concentrated in substantia nigra's zona compact a which becomes most damaged in Parkinson's disease. When iron reaches such higher concentrations in cells it can escape buffer control by ferritin and other iron binding proteins which is then catalytically convert hydrogen peroxide to generate highly reactive hydroxyl radical, which can damage DNA, lipids and biomolecules.

- High activities of two MAO-A and MAO-B which function to degrade dopamine into products that include hydrogen peroxide.
- High content of Melanin is one of the factor contributes to oxidative stress.
- Low GSH level in SN compared to other brain regions.[28]

An imbalance between the production and elimination of reactive oxygen species could contribute to the pathogenesis of Parkinson's disease and other neurodegenerative disorders. Metabolism of DA leads to the formation of several cytotoxic molecules, including superoxide anions ($O_2^{\cdot -}$), dopamine–quinone species (SQ·) and hydroxyl radicals (OH·). In PD, however, an abnormal increase in the production of reactive oxygen species might tilt the balance between production and elimination, leading to enhanced oxidative stress. DOPAC,3,4-dihydroxyphenylacetic acid MAO, monoamine oxidase.

$$DA + O_2 + H_2O \xrightarrow{MAO} DOPAC + NH_3 + H_2O_2$$

$$DA + O_2 \longrightarrow SQ^{\cdot} + O_2 + 2H+$$

$$DA + O_2^{\cdot -} + 2H+ \longrightarrow SQ^{\cdot} + H_2O_2$$

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH^{\cdot} + OH^{-} + Fe^{3+}$$

Oxidative process is intimately linked to other components of the degenerative process such as

- Mitochondrial dysfunction
- Excitotoxicity
- Nitric oxide toxicity
- Inflammation

Mitochondrial dysfunction:

Mitochondria are central to the generation of reactive oxygen and nitrogen species and integration of pro and anti-apoptotic signals in the cell.[29] It also acts as caspacious sink for Calcium homeostasis. The brain utilizes oxidative phosphorylation for generating ATP, which occurs in the inner mitochondrial membrane by a series of coupled redox reactions. Complex I-IV are present in inner mitochondrial membrane. During phosphorylation free radicals are produced from the transfer of a single electron to oxygen to generate superoxide anion. Superoxide anion is the proximal mitochondrial ROS mainly produced in the mitochondrial matrix, where it is rapidly converted to hydrogen peroxide catalyzed by Mn -SOD. In the presence of metal ions such as Fe^{2+}, hydrogen peroxide can be converted to the highly reactive hydroxyl radical (Fenton reaction). Complex I of the mitochondrial membrane is the main site of free radical production. The conditions favoured ROS production at complex I [30]

- ✓ Low ATP production and a reduced ubiquinone pool.
- ✓ High NADH/NAD+ ratio in the matrix.

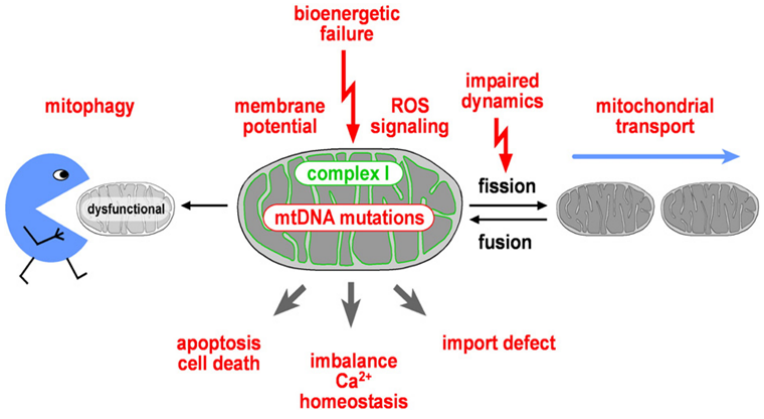


FIG.NO:2

Excitotoxicity:

Oxidative phosphorylation is utilized for producing energy in the brain. Impairment of oxidative phosporylation will enhance vulnerability to excitotoxicity.[31]Substantia nigra neurons possess NMDA receptors and there are glutamergic inputs from both cerebral cortex and subthalamic nucleus. Inaddition subthalamic neurons provide excitatory innervations to dopaminergic neurons in the substantia nigra pars compact a contain glutamate receptors. After activation of excitatory aminoacid receptors there is an influx of calcium followed by activation of nitric acid synthese leads to generation of peroxynitrate. It produces excitotoxic damages in substantia nigra pars compacta.

Nitric oxide toxicity:

Peroxynitrite appears to be an important factor in NO induced cell toxicity. When cells are under oxidative stress and unable to extinguish extra reactive oxygen species (ROS), which will accumulate in the cells, react with NO, and form peroxynitrite can further react with other compounds, produce more toxic peroxide products, cause DNA damage and activate caspase dependent and/or independent cell death pathways.[32] NO and peroxynitrite-mediated DNA damage and subsequent over activation of poly (ADP-ribose) polymerase-1 (PARP-1) are key pathways leading to cell death.[33] Over activation of PARP may deplete nicotinamide adenine dinucleotide (NAD^+) and ATP, leading to a major energy deficit and cell death and also can induce the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, and AIF is the key executioner in PARP-mediated cell death.[34]

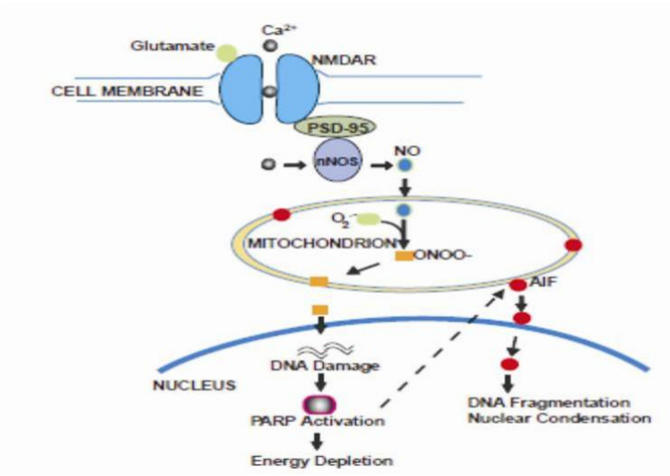


FIG.NO:3

Inflammation:

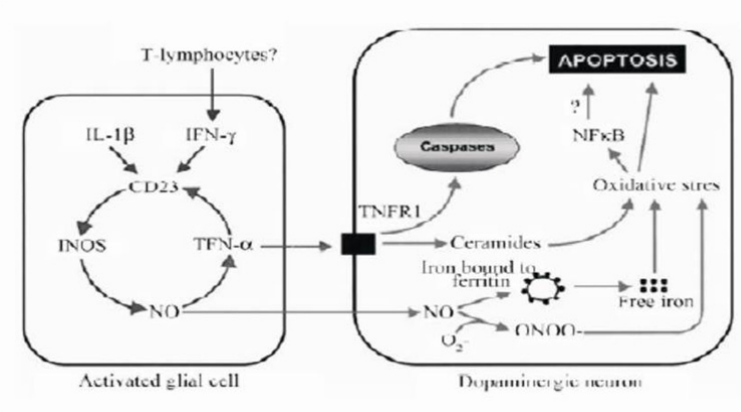


FIG.NO:4

Putative deleterious role of neuroinflammatory processes in Parkinson's disease (PD):

Proinflammatory cytokines, including IL-1β, TNF-α and IFN-γ, induce CD23 expression in glial cells whose engagement (by a ligand as yet to be identified) triggers iNOS expression and NO release. NO may amplify the production of cytokines within the glial cells but also diffuse to neighboring dopaminergic neurons. Of note, it is still debated whether infiltrated T lymphocytes could be the cellular source of IFN-γ in PD brain. The pathway shown in dopaminergic neuron possible inflammatory-associated cytotoxic mechanisms in dopaminergic neurons. NO produced by activated glial cells can react with superoxide ($O2^{-}$) to form peroxynitrite ($ONOO^{-}$), which can damage proteins and other cell constituents. NO also may contribute to oxidative stress by releasing iron from ferritin. Alternatively, cytokines may activate receptors (*e.g.* TNFR1) coupled to death signaling pathways. These pathways may involve activation of caspases and/or an oxidant-mediated apoptogenic mechanism through the release of ceramide and the activation of the transcription factor NFκB.[35]

Glutathione depletion:

Glutathione is a potent molecular antioxidant and an essential cofactor for the glutathione peroxidase family of antioxidant enzymes. Its depletion contribute to neurodegenerative disorders. GSH depletion could arise due to genetic propensity, poor diet, pharmaceutical treatment (use of acetaminophen) and function of ageing. The reduction in GSH may impair H_2O_2 clearance and promote OH formation, particularly in the presence of increased iron. At the same time significant increase in the level of γ-glutamyltranspeptidase (γ-GTT-the enzyme responsible for translocation of glutathione precursors and metabolism of oxidized form of glutathione)[36] which recruit glutathione precursors into cells to replenish diminished levels of GSH.[37]

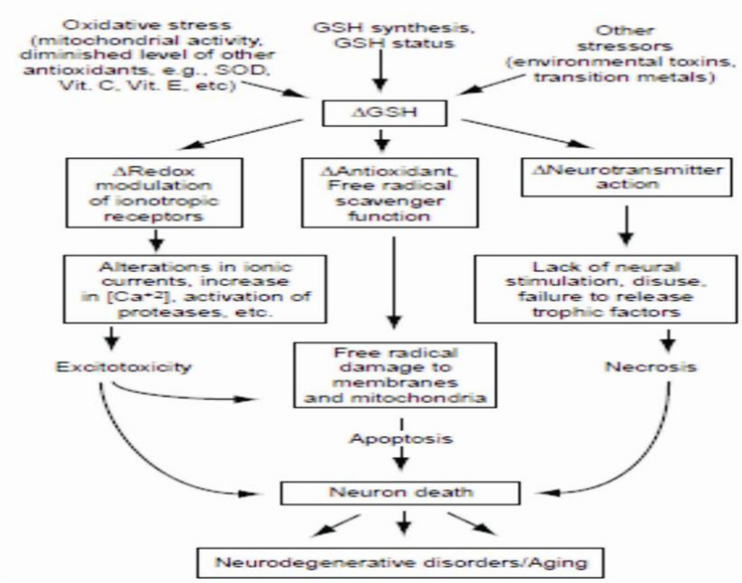


FIG.NO:5

Nutritional deficiency:

The brain uses the same nutrients that other organs use. Therefore all nutrient classes are useful to Parkinson's disease. Certain individual aminoacids are precursor to brain neurotransmitters and significantly ameliorate symptoms when given as dietary supplements. L-methionine is an essential aminoacid which may benefit in

Parkinson's disease. A number of B-vitamins, Vitamin C and E may also benefit in Parkinson's disease.[38]

Metals:

Metals such as iron can promote OH formation and catalyze the transformation of ∝-synuclein to aggregates. Elevated level of iron present in PD substantia nigra.

$$O_2 + Fe^{2+} \rightleftharpoons O_2^- + Fe^{3+}$$

$$H_2O_2 + Fe^{2+} \rightleftharpoons OH^{\cdot} + OH^- + Fe^{3+}$$

CLINICAL FEATURES:

Prototypical features of Parkinson's disease include [39]

a. Bradykinesia

b. Tremor

c. Rigidity

d. Postural instability

It includes various motor symptoms and non motor symptoms.

Motor symptoms:

- ✓ Dysarthria
- ✓ Dysphagia
- ✓ Hypomimia
- ✓ Hypophonia
- ✓ Micrographia

Non motor symptoms

Autonomic dysfunction

- Hypotension
- Bowel &bladder dysfunction

Sensory disturbances

- Pain
- Paresthesia

Mental status changes

- Confusional state
- Dementia
- Psychosis
- Sleep disturbances

NEUROTRANSMITTER AND RECEPTORS RELATED TO PARKINSON'S DISEASE

Dopamine:

It is a prototypical slow neurotransmitter that plays significant role in a variety of not only motor functions but also cognitive, motivational, and neuroendocrine.[40]

Distribution of dopamine

The distribution of the dopamine in the brain is more restricted. It is abundant in the Corpus striatum, a part of the extrapyramidal system concerned with the co-ordination of the movement and high concentration occurs in certain parts of the limbic system and hypothalamus.

Synthesis and metabolism

Dopamine, a catecholamine is synthesized in the terminals of dopaminergic neurons from tyrosine and transported for storage in the synaptic vesicle until stimulation to release into synaptic cleft. Dopamine activity is terminated by reuptake into presynaptic neurons by a transporter called *Dopamine transporter.* Catabolic pathways involve monoamine oxidase or Catachol - O – methyl transferase.[41]The main products are *Dihydroxyphenylacetic acid* and *Homovanillic acid.* The brain content of Homovanillic acid is an index of *dopamine turnover.*

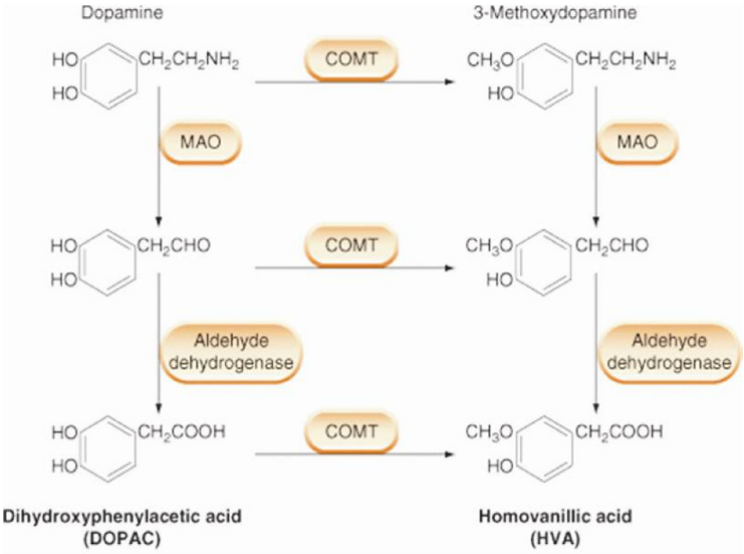


FIG.NO:6

DOPAMINERGIC PATHWAYS IN CNS AND ITS FUNCTIONS

Dopaminergic neurons projects from the pars compacta of the substantia nigra to the striatum via nigrostriatal pathways.

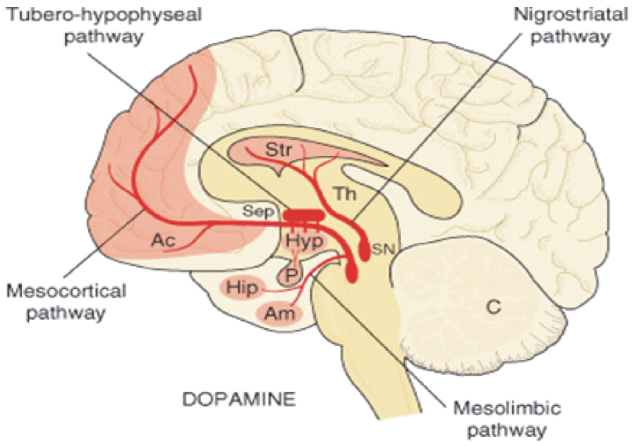


FIG.NO:7

There are three dopaminergic pathways

1. Nigrostriatal pathway, involved in motor control.
2. Mesolimbic/mesocortical pathways, running from group of cells in the midbrain to the part of the limbic system especially the nucleus accumbens, and amygdaloid nucleus and to the frontal cortex.
3. Tuberohypophyseal system is a group of short neurons projecting from ventral hypothalamus to the median eminence and pituitary, the secretion of which regulate.

Dopamine receptors

On the basis of biochemical, pharmacological and physiological criteria, DA receptors have been classified into two groups, termed D1 and D2.[(42)] Genes encoding members of the DA receptor family are part of a larger superfamily of genes comprising the G protein-coupled superfamily receptors (GPCRs).[(43)] D1 family consists of D_1 and D_5 while the D2 family which is more important in CNS function consists of D_2, D_3, D_4.

Distribution:

Dopamine receptors are expressed in the brain in distinct but overlapping areas

D_1 - is most abundant and widespread in areas receiving dopaminergic Innervations. (namely the striatum, limbic system, thalamus and hypothalamus).

D_2 - occurs in the striatum, Substantia nigra pars compacta, pituitary gland.

D_3 - occurs in olfactory tubercle, nucleus accumbens and hypothalamus.

D_4 - Distributes mainly in the central cortex, Medulla and Midbrain.

D5 - Distributes mainly in hypothalamus and striatum.

D_1 and D_2 are linked to activation and inhibition of adenyl cyclase activity.

PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

The basal ganglia are located in the basal telencephalon and consist of five interconnected nuclei: the caudate nucleus, putamen, globus pallidus, substantia nigra and subthalamic nucleus. It has specific patterns of activation in the initiation, sequency and modulating of motor activity.

Functional organization of Basal ganglia:

The striatum, the main input nucleus of the circuit transmits the flow of information received from the cortex to the basal ganglia output nuclei, substantia nigra pars reticulata and medial globus pallidus, via a direct and an indirect pathway. The two pathways originate from different subsets of striatal neurons viz direct and an indirect pathway. In the direct pathway, striatal GABA ergic neurons, containing dynorphin as a co-transmitter and expressing D_1 dopamine receptors, project mono-synaptically to the substantia nigra pars reticulata and medial globus pallidus. In the indirect pathway, the striatal output reaches the target nuclei via a more complicated route. In fact different subset of GABAergic neurons containing enkephaline and expressing D_2 receptors project to the lateral globus pallidus, which sends GABAergic projections to the subthalamic nucleus. The subthalamic nucleus, in turn, sends its glutamatergic efferents to the output nuclei and to the lateral globus pallidus. From the output nuclei, inhibitory, GABAergic projections reach the ventral lateral and ventral anterior nuclei of the motor thalamus. Thalamic nuclei then send glutamatergic projections to the motor cortex, thus closing the loop.

The activation of the direct or the indirect pathway leads to opposite changes in the net output of the basal ganglia circuitry. In fact, activation of the striatal GABAergic neurons that give rise to the direct pathway causes inhibition of GABA-ergic neurons of the output nuclei. This leads to disinhibition of thalamic nuclei, which are under the inhibitory control of the output nuclei projections.Conversely, activation of the striatal neurons that project to the lateral globus pallidus, in the indirect pathway, causes inhibition of the lateral globus pallidus and subsequent disinhibition of the subthalamic nucleus. The activation of the subthalamic nucleus which is glutamatergic increases the activity of the output nuclei. Consequently, their inhibitory control over the motor thalamus results enhanced.[44]

Neurochemical changes involved in Parkinson's disease

The neurodegenerative process of PD causes a functional re-arrangement of the basal ganglia circuitry. The dopaminergic denervation of the striatum triggers a cascade of events that leads, ultimately, to the increased activity of basal ganglia output nuclei. Enhanced activity of the output nuclei would be the result of enhanced glutamatergic drive from the subthalamic nucleus. The model also predicts that the enhanced activity of the output nuclei results in an increased inhibitory control over the motor thalamus and subsequent reduction of the thalamic glutamatergic output to the motor cortex. These changes are thought to represent the neural substrate for parkinsonian motor symptoms.[45,46]

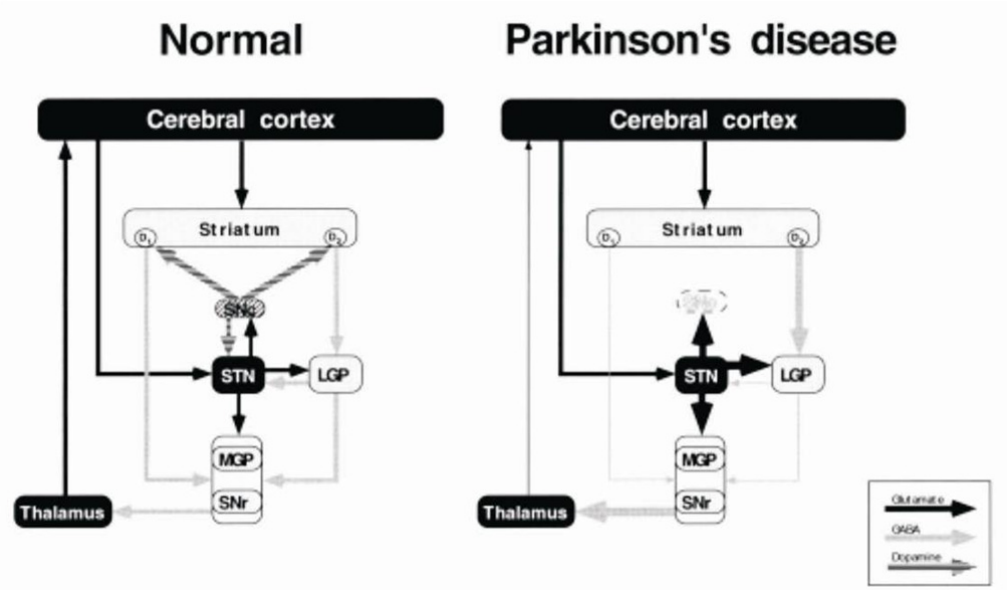


FIG.NO:8

NEUROPATHOLOGY OF PARKINSON'S DISEASE

The pathological hallmarks of Parkinson's disease are round eosinophilic intracytoplasmic proteinacious inclusions termed Lewy bodies (LBs) and dystrophic neuritis present in surviving neurons. In PD nirostriatal pathway degenerates.As a result marked loss of dopaminergic neurons that project to the putamen and much more loss of those project to caudate (thin red line).[19]

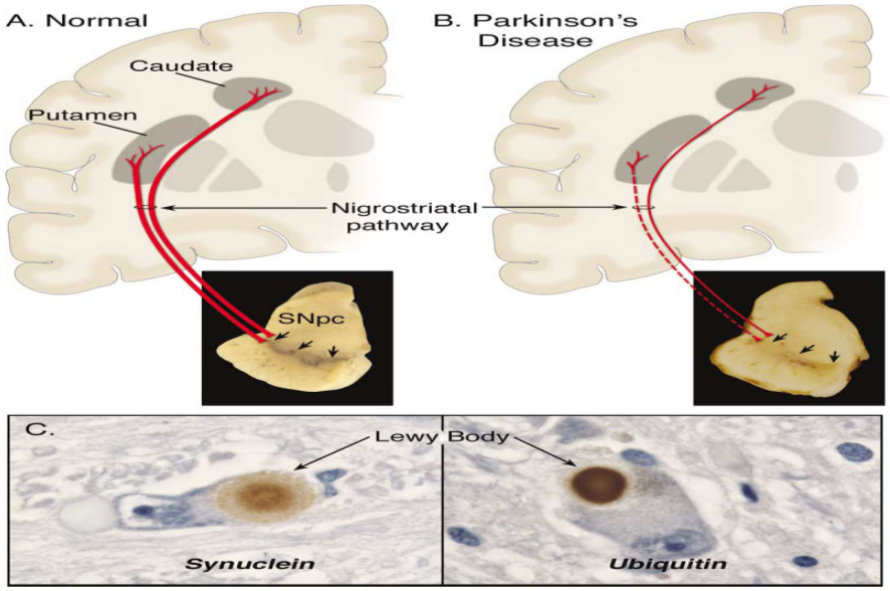


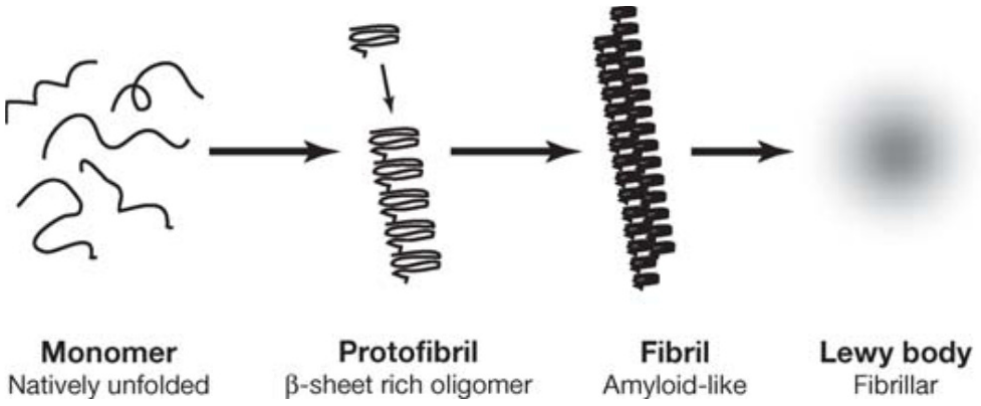
FIG.NO:9

The familial PD linked genes, responsible for pathogenesis are α-synuclein, Ubiquitin C-terminal hydrolase L1 (UCHL1), Parkin, PINK I and a newly identified gene known as DJ-1. Mutations in α-synuclein and UCHL1 are linked to autosomal dominant familial PD, while mutations in parkin and DJ-1cause autosomal recessive PD (ARPD).

α-SYNUCLEIN IN PARKINSON'S DISEASE

α-synuclein is a 140 aminoacid protein consists of a N –terminal amphipatic region containing six imperfect repeats (with a KTKEGV consensus motif), a hydrophobic central region containing non-amyloid β component domain and an acidic terminal region. It is intrinsically unstructured or native unfolded protein which has significant plasticity. It is highly expressed throughout the mammalian brain and

is enriched in presynaptic nerve terminals, where it can associate with membranes and vesicular structures. *α-synuclein* is considered to play a central role in the pathophysiology of PD. Two missense mutations in A30P and A53T in alpha synuclein display an increased propensity to self-aggregate to form oligomeric species. The A53Tand A30P mutations both share the capacity to promote the oligomerization, but not fibrillization, of *α*-synuclein.[22]Catecholamines, particularly dopamine, can react with *α*-synuclein to form covalent adducts that slow conversion of protofibrils to fibrils.Fibrillar forms of the *α*-synuclein protein as a major structural component of LBs in PD.[(47)]



Alpha synuclein fibrillogenesis

FIG.NO:10

PARKIN:

It encoded by a PARK2 gene. The *parkin* gene encodes a 465-amino-acidprotein with a modular structure that contains an N-terminal ubiquitin-like (UBL) domain, a central linker region, and a C-terminal RING domain comprising two RING finger motifs separated by in-between-RING (IBR) domain parkin can function as an E3 ubiquitin protein ligase.[23] E3 ligases are an important part of the cellular machinery that covalently tags target proteins with ubiquitin. Ubiquitination of proteins results from the successive actions of ubiquitin-activating (E1), conjugating (E2), and ligase (E3) enzymes resulting in the formation of a poly-ubiquitin chain containing four or more ubiquitin molecules. Such poly-ubiquitinated proteins are specifically recognized by the 26S proteasome and are subsequently targeted for

degradation. Mutation in the parkin gene results in the failure of ubiquitin proteosomal system for degradation of proteins which finally leads to cell death.

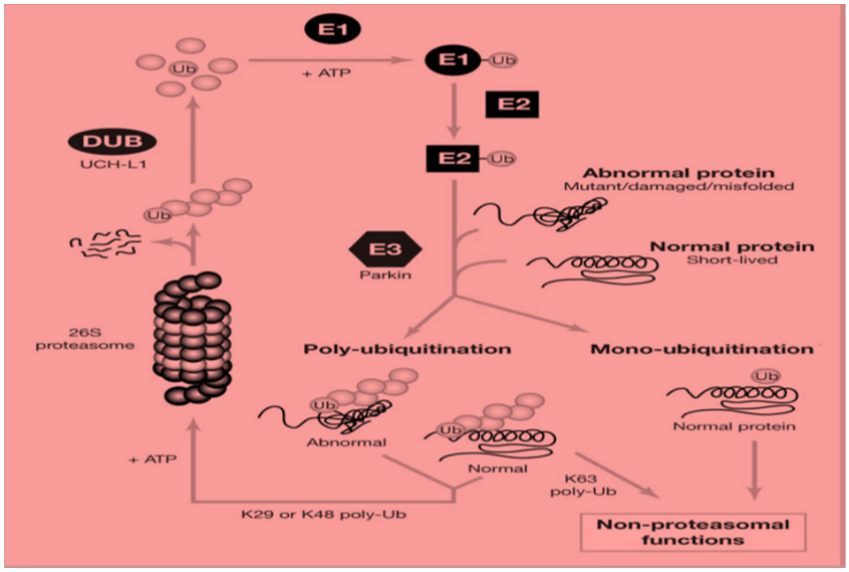


FIG.NO:11

THE UBIQUITIN-PROTEASOME SYSTEM:

Ubiquitin (Ub) monomers are activated by the Ub-activating enzyme (E1) and are then transferred to a Ub-conjugating enzyme (E2). Normal or abnormal target proteins are recognized by a Ub protein ligase (E3), such as parkin, which mediates the transfer of Ub from the E2 enzyme to the target protein. The sequential covalent attachment of Ub monomers to a lysine (K) acceptor residue of the previous Ub results in the formation of a poly-Ub chain. Poly-Ub chains linked through K29 or K48 signal the target protein for degradation through the 26S proteasome in an ATP-dependent manner, resulting in the generation of small peptide fragments. The resulting poly-Ub chains are recycled to free Ub monomers by deubiquitinating (DUB) enzymes, such as UCH-L1, for subsequent rounds of ubiquitination. The addition of Ub also has other diverse roles. Normal protein can be singly or multiply mono-ubiquitinated, or poly-ubiquitinated with K63-linked chains, which lead to non proteasomal functions that include DNA repair, endocytosis, protein trafficking, and transcription.[48]

UBIQUITIN C- TERMINAL HYDROLASE L1 (UCHL1)

UCHL1 belongs to the family of deubiquitinating enzyme, abundantly expressed in the brain (about 1% of total brain protein) and its expression is highly specific to neurons and to cells of endocrine lineage.The function of UCHL1 is that hydrolysis of small C-terminal adducts-ubiquitins which is important in proper functioning of Ubiquitin-Proteosome system.

Mutation of UCHL-1 leads to aberrations in proteolytic pathways and aggregation of proteins in Lewy bodies.[19]

PINK1:

PINK1 is a 581-amino-acid protein that contains a mitochondrial targeting sequence at its N-terminus and a highly conserved protein kinase domain. PINK1is considered to be a mitochondrial protein kinase, phosphorylates mitochondrial proteins, in response to cellular stress, to prevent mitochondrial dysfunction.[(49)] Mutation in PINK1 causes the loss of the putative kinase activity of PINK1 that affects mitochondrial function.

DJ1

Mutations in DJ-1cause autosomal recessive PD (ARPD). DJ-1 is more relevant to PD. Pathogenesis is its putative function as an antioxidant protein.

COMMON PATHWAYS UNDERLYING PD PATHOGENESIS:

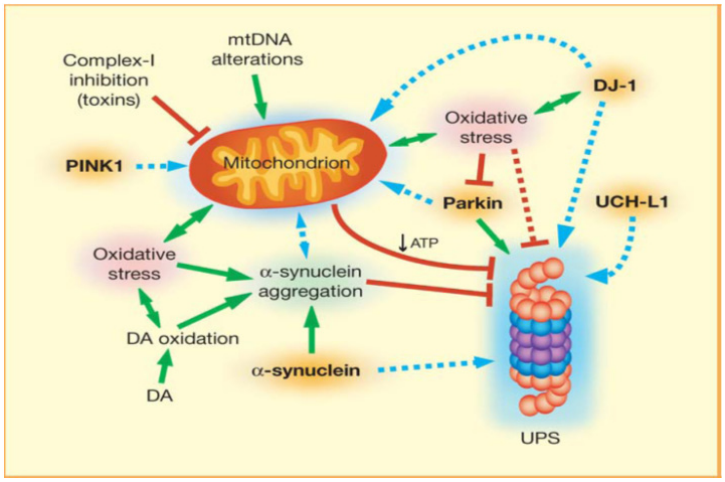


FIG.NO:12

Mutations in five genes encoding *α-synuclein*, *parkin*, *UCHL1*, *PINK1*, and *DJ-1* are associated with familial forms of PD through pathogenic pathways that may commonly lead to deficits in mitochondrial and UPS function. PINK1, parkin, and DJ-1 may play a role in normal mitochondrial function, whereas parkin, UCH-L1, and DJ-1 may be involved in normal UPS function. *α*-synuclein fibrillization and aggregation is promoted by pathogenic mutations, oxidative stress, and oxidation of cytosolic dopamine (DA), leading to impaired UPS function and possibly mitochondrial damage. *α*-synuclein may normally be degraded by the UPS. Some environmental toxins and pesticides can inhibit complex-I and lead to mitochondrial dysfunction, whereas alterations in mitochondrial DNA (mtDNA) may influence mitochondrial function. Impaired mitochondrial function leads to oxidative stress, deficits in ATP synthesis, and *α*-synuclein aggregation, which may contribute to UPS dysfunction. Oxidative and nitrosative stress may also influence the antioxidant function of DJ-1, can impair parkin function through *S*-nitrosylation, and may promote dopamine oxidation. Excess dopamine metabolism may further promote oxidative stress. Mitochondrial and UPS dysfunction, oxidative stress, and *α*-synuclein aggregation ultimately contribute to the demise of DA neurons in PD.Red lines indicate inhibitory effects, green arrows depict defined relationships between components or systems, and blue dashed arrows indicate proposed or putative relationships.[(50)]

STAGES OF PARKINSON'S DISEASE

Stages of Parkinson's disease are of five.[51]

Stage 1:

- Signs and symptoms on one side only
- Symptoms mild
- Symptoms inconvenient but not disable
- Usually presents with tremor of one limb
- The noticed changes in posture, locomotion and facial expression

Stage 2:

- Symptoms are bilateral
- Minimal disability
- Posture and gait affected

Stage 3:

- Significant slowing of body movement
- Early impairment of equilibrium on walking or standing
- Generalised dysfunction that is moderately severe

Stage 4:

- Severe symptoms
- Can still walk to a limited extent
- Rigidity and bradykinesia
- No longer able to live alone
- Tremor may be less than early stage

Stage 5:

- Cachetic stage
- Invalidism complete
- Cannot stand or walk
- Require constant nursing care

DIAGNOSIS OF PARKINSON'S DISEASE

There is no single cause method to make a positive diagnosis of Parkinson's disease the following are somewhat help to diagnose Parkinson's disease.

1. Neuroimaging
2. Olfactory system testing
3. Autonomic system testing

NEUROIMAGING

In this Single Photon emission Tomography is used along with radiolabelled compound. The compound will bind on to dopamine receptors and can be viewed using SPECT.This method allows the measurement of amount of dopamine releasing neurons.

OLFACTORY TESTING

In this the patient has to smell a variety of odours and then making a choice from a variety of possible answers for each one.

AUTONOMIC SYSTEM TESTING

Testing involves examining breathing, heart rate, reflexes and thermoregulation.

TREATMENTS

There is no single, optimal treatment for disease.Currently available therapies either boosts the levels of dopamine in brain or mimic the effects of dopamine.[52,53]

Levodopa:

Levodopa has been the mainstay of pharmacological treatment for Parkinson's disease. It is the metabolic precursor of dopamine, crosses the blood brain barrier by a large neutral amino acid transporter and is capable of reaching the striatal tissue where it is decarboxylated to dopamine.Taken alone it causes nausea and undergoes rapid metabolism by peripheral decarboxylase. To overcome this limitation it should be given along with dopa decarboxylase inhibitor Carbidopa. Levodopa decreases the rigidity, tremors and other symptoms of Parkinson's disease. The daily dose of L.dopa depending on symptoms and severity of side effects. L. dopa and carbidopa given as combined tablets (sinimet). On long term therapy causes motor fluctuations and dyskinesia occur in most patients.

Mono Amine Oxidase B inhibitors:

Eg: Selegiline, Rasagline.

Selegiline, the agent for symptomatic treatment of parkinson's disease prolongs the half life of endogenously produced dopamine by retarding the breakdown of dopamine in the striatum which benefit the patients of parkinsonism. Adverse effects such as Involuntary movements, postural hypotension, nausea, confusion and psychosis.

Rasagiline is restricted analog of selegiline and is a newly approved compound for treatment of PD.It has MAO- B inhibitory activity.

Muscarinic receptor antagonists:

These are useful in the management of mild to moderate symptoms of the drug induces parkinsonism. Trihexphenidyl or Benztropine are specially against tumor. In addition to it also reduces bradykinesia. Dryness of mouth, hallucination, confusion, agitation. Increased sensitive to dementia are major limitations.

Catachol - O - Methyl transferase inhibitors:

Eg: Tolcaptone, entacapone.

These are act by inhibiting catachol- o-methyl transferase and reduce central, peripheral metabolic degradation of L dopa. Hypotension, abdominal pain, diarrhoea, urinary discolouration, dyskinesia are major side effects.

Dopamine releasers:

Eg: Amantadine

It inhibits the activity of NMDA receptors and it promotes the release, prevents reuptake or have an influence the synthesis of dopamine It produces cardiovascular disorders and also induces seizures. It also produces restlessness, depression, confusion and hallucinations.

Ergot derivatives - Bromocryptine, Pergolide, Lisuride, Cabergoline.

Non Ergot derivatives- Ropinrole, Pramiprexole, Apomorphine, Pirebedil. Both act by specific D_2 alone. They may be used alone to delay the need for Levodopa.

Novel therapeutic approaches:

GDNF:

It has ability to protect degenerating dopamine neurons in PD as well as promote regeneration of nigrostiatal dopamine system.But there is a very little evidence to support its widespread use.

The treatments under investigation:

Eg: Adenosine antagonists, alpha 2 adrenergic receptor antagonists.

LIMITATIONS OF CURRENT THERAPIES:

At highly progressed condoned of Parkinson's disease; the therapy may be minimal of use.Hence these agents are adequately soluble when administer via this route so, various techniques designed to improve the effectiveness of transversal drug delivery and developing more soluble and effective drugs.

NEWLY RESEARCHED NEUROPROTECTIVE AGENTS

Antioxidants play an important role in neuroprotective therapy.Antioxidant such as Tocopherol and Tocotrienols, Ascorbate, Vitamin D and vitamin E and minerals such as Zinc and selenium.[54,55]

Curcumin

- It has free radical scavenging activity.
- Potent inhibitor of peroxy nitrate and lipid peroxidation by enhancing the production of glutathione and protects neurons from degeneration.

A-Lipoic acid

- Alpha lipoic acid and its reduced form, dihydrolipoic acid (DHLA) also enhance cellular glutathione production.
- It also neutralise the hydroxyl radical, singlet oxygen hypochlorite, nitric oxide radicals and hydrogen peroxide.

Melatonin

- It is a powerful neuroprotectant.
- It acts as a free radical scavenger which reacts with hydroxyl radical, hydrogen peroxide, singlet oxygen, peroxynitrite, nitric oxide and hypochlorous acid.
- It stimulates the production of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase.

Coenzyme Q-10

- It can act by regenerating Vitamin-E which inturn stimulate α-Lipoic acid which can increase the level of Ubequinol, an agent for protecting oxidative stress.

Vitamin A, C AND E

- Lipid peroxidation is increased in the substantia nigra of patients with Parkinson's disease, suggesting that excess of free radicals contributes to nigral striatal neurodegeneration.
- Vitamin A, C&E are all proven antioxidants capable of preventing lipid peroxidation by acting as free radical scavenger.

OTHER AGENTS

- Flavanoid polyphenols such as epigallocatechin 3-gallate from green tea and quercetin from apples.
- Non flavanoid polyphenols such as resverastrol from grapes.
- Phenolic acids or phenolic diterpenes such as rosmarinic acid or carnosic acid from rosemary.
- Organosulphur compounds such as isothiocyanate,L-sulphoraphane from broccoli,and thiosulphonate allicin from garlic.
- Mucuna pruriens contain levodopa.But its clinical efficacy cannot be proven.It is under double blind trial.
- Ghrelin: Its function is to preserve the production and release of dopamine by substantia nigra.

Synthetic drugs

Caffeine, Cannabis, L- Carnitine.

NON PHARMACOLOGICAL TREATMENT UNDER INVESTIGATION

New drug delivery system for Parkinson's disease are increasingly more focused on site specific delivery of Pharmaceuticals. It includes the delivery of various newly researched drugs, genes, viruses and various peptides.[50]

Cell transplantation

- ❖ Transplantation of dopamine producing neurons to replace these degenerated neurons during the pathogenesis of Parkinson's disease is a promising approach to treatment.
- ❖ Hence it is the only advancement that has been shown the capacity to allow patients to achieve full restoration of their functional capacity.
- ❖ Grafts have shown minimal immunological rejection in recepients and in most successful trials have allowed patients to withdraw from Levodopa therapy.

LIMITATIONS

- ❖ Poor rate of graft cell survival was reported.

Gene therapy

- ❖ Neurologix's gene therapy is the only one gene strategy currently in development which by passes the dopamine system.
- ❖ In Parkinson's disease, patient's loss dopamine producing brain cells, resulting in substantial reduction in the activity and amount of GABA, the major inhibitory neurotransmitter in the brain, which contributes to abnormal increase in the activity of the STN of the brain.The gene responsible for GABA, is called glutamic acid decarboxylase.
- ❖ This involves restoring GABA and improving the patient's motor control.

LIMITATIONS

- ❖ However this technology remains in the experimental stages of development and in 2nd phase of clinical trials

Surgical methods

- Pharmacological therapy for Parkinson's become inadequate over long term use.
- Surgical interventions for Parkinson's disease have been shown to be beneficial for refractory symptoms.
- Thalamotomy and thalamic stimulations are considered as safe and effective procedures to treat tumors.
- Pallidotomy and pallidol stimulation primarily reduces dyskinesia.
- Currently Deep brain stimulation is the intervention of choice because this is more safer than other available techniques.

Table. 2:- **SURGICAL INTERVENTIONS OF PARKINSON'S DISEASE.**

Surgical intervention	**Description of Procedure**
Pallidotomy	It involves the use of an electric probe to destroy the small porting of the brain that is over active and thought to cause symptoms of PD.
Thalamotomy	It involves the removal of the thalamus in the brain .The thalamus is responsible for involuntary movements. It is rarely performed and only effective in providing relief from tumors.
Thalamic stimulation	Involves the insertion of an electrode wire into thalamus, the other end of which is connected to pulse generator under the skin in the thorax. The advantage of this procedure is that it can produce the benefit of thalamotomy without causing incision of the skin and demonstrated efficiency in the management of tremor in PD.
Deep brain stimulation	It is an alternative procedure used to destroy small regions of the brain .A thin electrode implanted into the brain prevents transmission of impulses for involuntary movements.[50]

ANIMAL MODELS FOR PARKINSON'S DISEASE

1. Acute Pharmacologic models[56]
Reserpine induced Parkinsonism
Haloperidol induced Parkinsonism
Ferric chloride induced Parkinsonism
Cholinomimetics induced Parkinsonism

2. Models exhibiting destruction of Dopaminergic nigrostriatal pathway
Surgical induction
6-OHDA model with partial lesion
6-OHDA model with full lesion

3. Pesticide induced model
Rotenone induced model
Paraquat induced model

4. Animal models based on hallmarks of PD
Proteosomal inhibitor models
Glial activation models

MPTP MODEL OF PARKINSON'S DISEASE

MPTP model of Parkinsonism is the most clinically relevant of all available models which mimics the clinical features of Parkinson's disease and also used for antiparkinsonism medications.

Mechanism of MPTP

MPTP, a neurotoxin that produces Parkinsonian syndrome in both humans and experimental animals'[57]

It is a highly lipophilic molecule crosses the blood brain barrier in a matter of seconds of systemic injection.

It is taken up into astrocytes where it is metabolized to MPP^+ by monoamine oxidase –B.

MPP^+ extruded from the astrocytes is taken up into mitochondria of dopaminergic neurons by DAT where it disrupts the oxidative phosphorylaton by inhibiting complex-1 site of mitochondrial electron transport chain.

This leads to impairment of ATP production, elevation in intracellular calcium levels and upregulation of TNF α, ILβ and nitric oxide synthase.

Upregulation of nitric oxide synthase increases the presence of nitric oxide.In the cytosol of dopamine neurons, nitric oxide reacts with superoxide dismutase to produce strong oxidant peroxynitrate which can damage cellular proteins, lipids and DNA, leads to neuronal damage.

Specific degeneration

MPTP treatment can induce the m-RNA expression of nNOS and guanyl cyclase beta subunit($GC\beta_1$) which leads to elevation in their protein levels and activated within the striatum and substantia nigra. These effects are accompanied by marked enhancement of C-GMP formation.7-Nitroindazole is used to decrease MPTP induced elevation in C-GMP levels.[58]

Poly (ADP-Ribose) polymerase is also involved in DNA plasticity such as repair of DNA damage, gene expression and carcinogenesis. Extensive PARP activation can promote cell death by energy depletion. Toxic effect of MPTP is mediated through excessive production of PARP. PARP inhibitors such as Benzamide are known to prevent MPTP induced neurotoxicity.

Neuroprotective factors produced after intoxication with MPTP prevent dopaminergic neurons from neuronal death by modulation of oxidative stress and

inflammation. GDNF is a potent neurotropic factor that has restorative effects in a wide variety of rodent and primate models of PD.

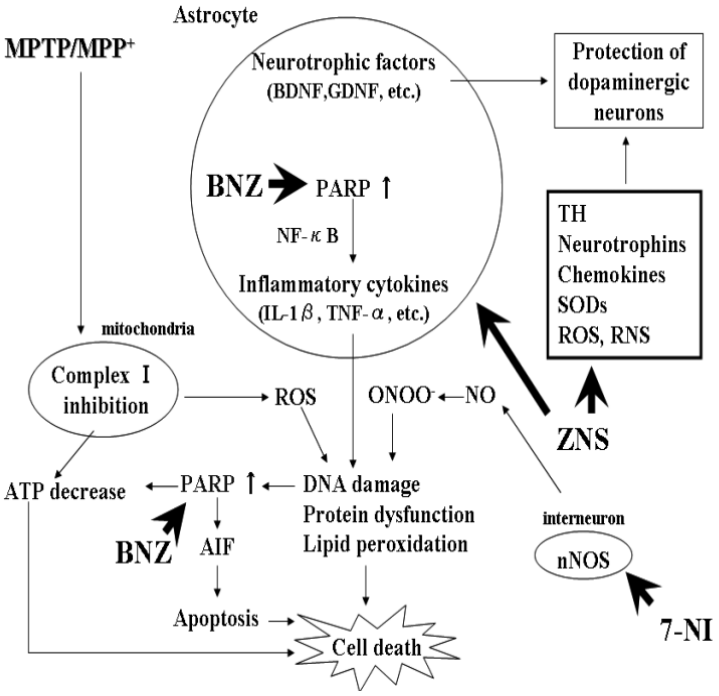


FIG.NO:13

OXIDATIVE STRESS AND EXCITOTOXICITY IN PARKINSON'S DISEASE

Oxidative stress is a major player in the pathology of Parkinson's disease. Oxidative stress damages nucleic acids, proteins and lipids and potentially opens the mitochondrial permeability transition pore which inturn can further stimulate ROS production, worsen energy failure and release proapoptotic factors such as cyochrome into the cytoplasm. Generation of high levels of ROS and downregulation of antioxidant mechanisms results in neuronal death.

The brain utilizes mitochondrial phosphorylation for energy production. Free radicals are side products of this process.The most common cellular free radicals are superoxide radical, peroxynitrate and hydroxyl radicals. When antioxidant system becomes overwhelmed by these free radicals oxidative damage and cell death can occur. Problems occur when ROS exceeds their elimination by the antioxidant protection system. The unbalance between cellular production of ROS and inability of cells to defend against these effects is called oxidative stress.

NO production increases in neurodegenerative disease as a consequence of oxidative stress. NO is activated by the release of glutamate combined with inhibition of glutamate removal which leads to NMDA receptor over activation and excess calcium influx.Excess of calcium influx is one of the cause of excitotoxicity and induce neuronal damage.[59]

LITERATURE REVIEW

1. Nagaraja Halaegrahara, et al.,**(2010)** investigated the neuroprotective effect of Centella asiatica extract against MPTP induced neurotoxicity in aged Sprague-dawley rats.For this study rats were divided into four groups such as control, *Centella asiatica* alone, MPTP alone (20mg/kg for 21 days) and MPTP with *Centella asiatica* (300mg/kg for 21 days).They demonstrated that datas from the evaluation of hippocampus homogenate shown MPTP challenged rats elicited a significant increase in Lipid peroxides (LPO) ($P<0.01$), PCC ($P<0.01$) and xanthine oxidase when compared with control rats. Furthermore, there is a significant decrease in total antioxidants (TA) ($P<0.001$), SOD ($P<0.001$), glutathione peroxidase ($P<0.01$) and catalase ($P<0.001$) level with MPTP treatment. These findings suggested that supplementation of CAE could reduce LPO and PCC significantly increased total antioxidant and antioxidant level in striatum and hippocampus. So CAE is effective in protecting the brain against neurodegenerative disorders such as Parkinsonism. [(60)]

2. M.Mohanasundari, et al.,**(2007)** evaluated the neuroprotective effect of *Hypericum perforatum* extract on the reaction of astrocytes in mice brain treated with an intraperitoneal injection of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg with 2 hr intervals). They suggested that treatment with Hypericum perforatum extract (HPE) resulted in an inhibition of monoamine oxidase-B (MAO-B) activity and reduced astrocyte activation in striatal area induced by MPTP. These results show that HPE has neuromodulating effect against MPTP induced Parkinson's disease in mice.[(61)]

3. Li-Xing Liu, et al.,**(2008)** investigated the neuroprotective effect of Genistein, an isoflavone naturally found in soya products on dopaminergic neurons in ovariectomized 1- methyl 4-phenyl 1,2,3,6 tetrahydropyridine induced PD model mice. They suggested that pretreatment with genistein significantly restore the level of dopamine, DOPAC and homovanillic acid and MPTP-induced down regulation of TH, dopamine transporter (DAT) and Bcl-2 mRNA expression in the midbrain in MPTP induced mice. Furthermore, MPTP-challenged with genistein group shown reduced neurotoxicity, with tyrosine hydroxylase-immunoreactive

(TH-IR) neurons in the substantia nigra pars compacta (SNpc) affected to a significantly lesser extent as compared to the MPTP treated control. These findings evidenced that genistein has neuroprotective effects on dopaminergic neurons in the MPTP-induced PD mice and this effect may be attributed to enhancing Bcl-2 gene expression.[62]

4. Linjuan Sun, et al.,**(2010)** investigated the effect of cysteamine against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced toxicity in the dopaminergic neurons in a mouse model for Parkinson's disease (PD). They suggested that pretreatment with low dose cysteamine (20 mg/kg/day) significantly reduce the loss of dopaminergic (DA) neurons and reduction in striatal DA concentrations and also reduce the increased production of pro-oxidants, such as reactive oxygen species (ROS) and malonidialdehyde and increased GSH level. In addition, the inhibited secretion of the brain derived neurotrophic factor (BDNF) by neurons derived from substantia nigra pars compacta(SNpc) of MPTP-treated mice was significantly restored by cysteamine administration. These results demonstrated that cysteamine at low dose confers potent neuroprotection against MPTP-induced toxicity of dopaminergic neurons, and may become a potential therapeutic strategy for PD.[63]

5. Angela B. Reksidler, et al.,**(2007)** investigated the effects of the selective cyclooxygenase-2 (COX-2) inhibitor parecoxib (Bextra™) in the prevention of motor and cognitive impairments observed in rats after an intranigral infusion of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a model of the early phase of Parkinson's disease. They suggested that treatment with parecoxib (10 mg/kg) administered prior to the surgery and daily (2 mg/kg) for the subsequent 21 days, prevented the MPTP-treated rats from presenting decreased locomotor and exploratory behavior, increased immobility, and impairment while performing the cured version of the Morris water maze. Furthermore, parecoxib treatment also significantly prevented the reduction of tyrosine hydroxylase protein expression in the substantia nigra (7, 14 and 21 days after surgery), and in the striatum (14 and 21 days after surgery) as immune detected by western blotting. These results strongly suggested that parecoxib exerts a neuroprotective effect on motor, tyrosine hydroxylase expression, and cognitive functions as it prevents their impairments within the confines of this animal model of the early phase of Parkinson's disease.[64]

6. Qing Zhao, et al.,**(2010)** investigated the effects of Echinacoside (ECH), a monomer extracted from herbs, on rescuing dopaminergic function in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-lesioned mice. We found that oral administration of ECH (30 mg/kg/day for 14 days) to MPTP-treated mice, commencing after impairment of the nigrostriatal system, suppressed the reduction of nigral dopaminergic neurons, striatal fibers, dopamine and dopamine transporter to 134.24%, 203.17%, 147.25% and 154.72% of MPTP-lesioned animals respectively($p<0.05$). There was a relative elevation in expression of GDNF and BDNF mRNA (2.94 and3.75-fold) and protein (184.34% and 185.93%) in ECH treated mice compared with vehicle treated MPTP lesioned mice ($p<0.05$). In addition, the apoptosis cells and Bax/Bcl-2 ratio of m RNA and protein in MPTP-lesioned mice significantly increased, and these effects could be prevented by ECH. At the 7th and 14th days of ECH treatment, the gait disorder displayed obvious improvement ($p<0.05$). These findings strongly demonstrated that ECH is probably a novel, orally active, non-peptide inducer of NTFs and inhibitor of apoptosis, and they provide preclinical support for therapeutic potential of this compound in the treatment of PD.[(65)]

7. Hua-Qing Liu, et al.,**(2006)** investigated, Paeoniforin attenuates neuroinflammation and dopaminergic neurodegeneration in the MPTP model of Parkinson's disease by activation of adenosine A_1 receptor. They explained subcutaneous administration of PF (2.5mg/kg and 5mg/kg)for 11 days could protect tyrosine hydroxylase positive substantia nigra neurons and striatal nerve fibres from death and bradykinesia induced by four dose injection of MPTP(20mg/kg)on day 8.These findings demonstrated that PF could reduce MPTP induced toxicity by inhibition of neuroinflammation by activation of A_1 and A_2 and suggested that PF might be a valuable neuroprotective agent for treatment of PD.[66]

8. Amit Gupta, et al., **(2010)**evaluated the neuroprotective effect of nimesulide, a preferential COX 2 inhibitor against 1 methyl-4-phenyl-1, 2, 3.6 tetrahydropyridine (MPTP) rat model of Parkinson's disease. They evaluated three parameters such as behavioural, biochemical and histological parameters for this study. Behavioural evaluation demonstrated that intrastriatal administration of MPTP (32Mmol in 2µl) produce a significant decrease in locomotor activity. Biochemical investigation of striatal region revealed that MPTP treated group

shown significant enhancement in oxidative stress in striatal region evidenced by increased lipid peroxidase levels, nitrate levels, myeloperoxidase activity along with depleted antioxidant pool and reduced redox (GSH/GSSG) ratio and also produce significant mitochondrial complex-I inhibition and reduction in mitochondrial viability. Histopathological examination of MPTP treated brain sections revealed some alteration in histoarchitecture. Furthermore, chronic administration of nimesulide at a dose of 5mg/kg p.o for 12 days reversed the MPTP induced effects. These findings strongly demonstrated that therapeutic value of COX inhibitors in treatment of neurodegenerative disorders such as Parkinson's disease.[67]

9. B.Han, et al.,**(2010)** found out the effect of Hydroxysafflor yellow A (HSYA) on MPTP induced neurotoxicity in mice. They suggested that pretreatment with hydroxysafflor at a dose of 2,8mg/kg for a week was followed by intraperitoneal injection with MPTP (30mg/kg) for five consecutive days. After that the subsequent behavior, biochemical index and immunohistochemical manifestations in mice were determined. Behavioral testing showed that MPTP-treated mice exhibited motor deficits but HSYA prevented the appearance of motor abnormalities. HSYA treated mice at dose of 8 mg/kg attenuated the reduction of dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and also the activity of SOD, catalase activity and GSH levels were significantly higher, compared to the MPTP-treated mice. These results indicated that HSYA possesses neuroprotective effects and is a promising anti-Parkinson's disease drug which is worthy of further study.[68]

10. Ying Jui Ho, et al.,**(2011)** evaluated the role of NMDA receptors in neuronal and behavioural changes in a 1 methyl -4 phenyl -1, 2, 3, 6- tetrahydropyridine disease. One day after intranigral infusion of MPTP causes transient disturbance in motor function which was evaluated by using Rotarod. MPTP lesioned rat's causes deficit in working memory and anxiety like behavior in elevated plus maze test and T maze test respectively. Furthermore, MPTP lesioned rats failed to recognize object, disrupted the level of interleukin 2 in striatum, amygdala and non-prefrontal cortex were increased and cell loss in the hippocampal CA1

area. These effects were improved by DCS treatment. These results suggested that NMDA receptors play a key role in PD-related neuronal and behavioural dysfunction.[69]

11. **Salman khan M, et al., (2013)** investigated chemotherapeutic potential of *Boerhaavia diffusa* linked from ancient time to the present with the scope in future. Furthermore a recent update on mechanistic approaches of *B. diffusa* has also been discussed. Based on antioxidant & antdiabetic characteristic it is hypothesized that *Boerhaavia diffusa* might exhibit antiglycating properties.[70]

12. **Kanjoormana Aryan Manu, et al., (2009)** evaluated Immunomodulatory activities of punarnavine, an alkaloid from *B. diffusa* using Balb/C mice. Punarnavine enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in presence and absence of specific mitogens in vitro and in vivo. More over administration of Punarnavine significantly reduced the LPS induced elevated levels of pro inflammatory cytokines such as TNF-α, IL-1β and IL-6 in mice.[71]

13. **Mandeep kaur, et al.,(2011)** investigated the methanolic extract of *Boerhaavia diffusa* roots & its different fraction including liriodendrin rich fraction for exploring the possible role of liriodend rinrich in its anticonvulsant activity. These finding concluded that observed anticonvulsant activity was due to calcium channel antagonistic action as this activity was retained only in liodendrin rich fraction which posse's significant anticonvulsant activity of liriodendrin in BAY K-8644 induced seizures.[72]

14. **Sandhya.k, et al., (2010)** had conducted a comparative study of hydro alcoholic extract and poly herbal formulation of *Boerhaavia diffusa* for their anti stress activity using cold restrainst stress model. Due to cold restrain stress there was imbalance in the level of biochemical parameter like glucose, triglycerides, cholesterol, SGOT, SGPT which were near normalized following the administration of HEBD & PHF-09. HEBD and PHF-09 were found to have comparable anti stress activity. [73]

15. **Surendar. k. Pareta et al.,(2011)** found out the effects of pre treatment of aqueous extract of *Boerhaavia diffusa* root(200-400 mg/kg /day) in repeated dose acetaminophen nephrotoxic rats for 14 days. Acetaminophen administration characterized by significant increase in Blood urea nitrogen (BUN), serum creatinine and increased level of kidney malondialdehyde prolein thiol, along with depletion of SOD, CAT, GPX and GSH. .Histopathological changes showed significant structural damage to kidney. The result suggest that *Boerhaavia diffusa* has the potential in preventing the acetaminophen induced Nephrotoxicity.[(74)]

16. **Ramachandran. y. L, et al.,(2010)** evaluated the hepatoprotective properties of petroleum ether extract, Methanolic extract and isolated compound of *B. diffusa* & *A. lanata* against carbon tetra chloride induced hepatic damage in rats. This study reveals that different dose of plant extract offer significant protection of serum test and liver histology.[(75)]

17. **Shisode. k.s, et al., (2011)** had studied that different extract of roots of *Boerhaavia diffusa* for invitro antioxidant activities & phytochemical screening. Among these there extract, ethanolic extract had shown better antioxidant activity & phytochemical screening revealed the presence of carbohydrate, saponins, proteins, flavonoids, steroids, fats & alkaloid**.**[(76)]

18. **Gopal. T.k, et al.,(2010)** evaluated invitro antioxidant activities of chloroform, ethanol & ethyl acetate fraction of *B. diffusa*. L which might have improved it's hepatoprotective action. The extract found to have significant Nitric oxide and DPPH radical scavenging activity. The result suggest that roots of *Boerhaavia diffusa* were found to reveal antioxidant potential which support the use of plant in traditional medicine.[(77)]

19. **Apurba sarker Apu. et al.,(2012)** investigated the bioactivities of crude n-hexane, ethyl acetate and methanolic extract of aerial parts of the *Boerhaavia diffusa* linn and its phytochemical analysis. Methanolic extracts showed higher anti oxidant, thrombolytic activity and less cytotoxic activity than that of n-hexane & ethyl acetate extract of *Boerhaavia diffusa*. All the extract showed significant inhibitory activity aqainst candida

albicans at a concentration of 1000µg/disc. These findings suggest that plant could be important source of medicinally important natural compound.[78]

20. **Shukla Anamika and Gupta Rakesh kumar, (2011)** studied the effects of aqueous extract of *Boerhaavia diffusa* roots and leaves on blood sugar level in Alloxan induced diabetic rats. These studies conclude that aqueous extract of *B. diffusa* have shown hypoglycemic effect may be due to presence of glycosides, flavonoids, tannins and saponin in the extract.[79]

21. **Suralkas A.A et al.,(2012)** investigated antihistamine activity of ethanolic extract of *Boerhaavia diffusa* linn roots using isolated goat tracheal chain and histamine induced bronchoconstriction in Guinea pig *Boerhaavia diffusa* significantly inhibited dose dependent contraction of goat tracheal chain produced by histamine and also showed significant protection by prolonging preconvulsion dyspnoea time in guinea pigs. Thus *Boerhaavia diffusa* showed antihistaminic and bronchodilating activity against histamine and hence posses potential role in treatment of asthma.[80]

22. **Surendran. k, et al., (2010)** evaluated anti urolithiatic activity of *Boerhaavia diffusa* lina root aqueous extract and rationalize it's use in treating renal stone. The lithogenic treatment causes weight loss, hyperoxalurea and impairment of renal function. *Boerhaavia diffusa linn* causes diuresis and hasten the process of dissolving crystals and helps in mechanical expulsion of stones and improve the renal function by removing the waste product and decrease oxalate excretion by interfering with metabolism. Results of this study indicate *Boerhaavia diffusa linn* posses antiurolithiatic that possibly mediated through diuretic and hypo-oxaluric effects.[81]

23. **Mahesh, A.R, et al., (2012)** had conducted a detailed study on *Boerhaavia diffusa* for it's medicinal importance. Various phytochemical ,pharmacological ,experimental and clinical investigation are done on *Boerhaavia diffusa.* This include evidence based over view of pharmacological, phytochemical properties of aerial parts & the roots of *Boerhaavia diffusa* ,which may be helpful to establish a standard natural drug for further studies.[82]

24. **Meena, A.K, et al., (2010)** investigated the standardized and phytochemically evaluated aqueous and hydroalcoholic extracts of *Boerhaavia diffusa*. It involve pharmacognostical examination of morphological and microscopical characters and phytochemical investigation of *Boerhaavia diffusa* including determination of loss on drying, ash values, TLC and extractive values. The qualitative chemical examination revealed the presence of various phytoconstituents like carbohydrate, ,saponins, phenolic compound and mucilage in the extract.[83]

25. **Babita Aqrawal,et al., (2011)** have investigated a review on it is phytochemical and pharmacological profile Phytochemical studies had shown the presence of rich source of alkaloids, steroids and flavones. pharmacological research explains hepatoprotective, diuretic, anti inflammatory ,anti-stress and immunomodulation anti fertility, antimicrobial, antiviral, and insecticidal activities. In conclusion *Boerhaavia diffusa* contain biologically active compounds that may serve as candidate for new drugs in the treatment and prevention of human live stock diseases.[84]

26. **Goyal.B.M et al., (2010)** analyzed an overview of pharmacological potential of *Boerhaavia diffusa*. It covers various physiology, pathology of disease and their therapies. This article includes evidence based information regarding pharmacological activity of this plant. It has many ethanobotanical users and is medicinally used in the traditional Ayurvedic system.[85]

27. **Bhavin, A, et al., (2013)** investigated the effect of hydro alcholic extract of roots of *Boerhaavia diffusa* in experimental Beningn prostatic hyperplasia in rats. Body weight, prostate weight, bladder weight and serum testosterone were measured and histological studies were carried out. The result suggested that treatment with *Boerhaavia diffusa* may improve symptoms of disease and inhibit the increased prostrate sign. Invitro study implies that herbal extract had a beneficial effect on prostatic smooth muscles which relieve the urinary symptom and disease.[86]

28. **Krishna murti, et al., (2001)** evaluated antidiabetic activity of ethanolic extract of roots of *Boerhaavia diffusa* against streptozocin induced experimental rats. Blood glucose level were determined on 0, 7th, 14, & 21st day after oral administration. The effect of ethanolic extract of B.diffusa on serum lipid profile like total cholesterol, triglycerides, LDl, VLDL, HDL were also measured in diabetic and non diabetic rats. The ethanolic extract of *Boerhaavia diffusa* was found to reduce blood sugar level in streptozocin induced diabetic rats. There was significant reduction in total cholesterol, LDL, VLDL, & improvement in HDL cholesterol in diabetic rats. The results indicated that *Boerhaavia diffusa* posses a hypoglyceamic & antihyperlipideamic effect.**(87)**

29. **Ajmire. P.V, etal.,(2011)** had conducted study of alcoholic & aqueous extract of whole plant of *Boerhaavia diffusa* against DMNO induced liver cirrhosis in rat's model. The activity was assessed using ILS, histopathological studies of liver, biochemical & heamatological studies. EEBD & AEBD shows significant increase in survival time, a decrease in cirrhotic nodules. The biochemical & heamatological parameter were also corrected by EEBD & AEBD in DMN induced rats. However out of these two extract, EEBD shows maximum anti cirrhotic effect than AEBD.**(88)**

30. **Venkatesh. P, et al.,(2012)** evaluated analgesic & antipyretic activity of various doses of alcoholic extracts of stem & leaves of *Boerhaavia diffusa* & leaves of Anisochilus carnosus. Tail immersion method & Hot plate in mice were studied for analgesic activity. Alcoholic extract of *Boerhaavia diffusa* had shown significant analgesic & antipyretic activity.**(89)**

31. **Mohammed Khalid, et al., (2012)** had studied pharmacological evalution and qualitative analysis of *Boerhaavia diffusa L*. root. Various parameters like macroscopy, microscopy, fluorescence analysis as well as extraction value and qualitative phytochemical screening of different extraction were studied. The major components of extractions like total phenolic, total flavonoids were also estimated.**(90)**

32. **Venkatesh. P, et al., (2012)** evaluated a study on alcoholic extract of stem and leaves of *Boerhaavia diffusa* and leaves of Anisochilus carnosus on CCL4 induced hepatotoxicity in rats. Different dose levels administered. Biochemical parameters of liver like SGOT, SGPT, serum alkaline phosphatase, total and direct serum bilurubin were determined. It was concluded that the alcoholic extract of AEBD and AEAC possess hepato protective activity against CCL4 induced hepatotoxicity in rats.[91]

FOCUS OF THE PRESENT STUDY

The present study was undertaken to find out the potential activity of *Boerhaavia diffusa* against breast cancer and their effects on MPTP induced breast Neurodegenaration in rats.

From time immemorial mankind's efforts and ultimate aim have been to seek eternal happiness. And his endeavour has been to overcome and seek appropriate remedies for things that stand in his way. Plants have played a weighty role in maintaining human health and improving the quality of human life for thousands of years and have several precious components of medicines, seasonings, beverages, cosmetics, and dyes. Herbal medicines are based on the premise that plants contain natural substance that can promote health and alleviate illness. In recent times focus on plant research has increased all over the world and large evidence has collected to show immense potential of medicinal plants used in various traditional systems.

Today we are witnessing a great deal of public interest in the use of herbal remedies. Many western drugs had their origin in plant extract. There are many herbs, which are preponderantly used to treat cardiovascular problems, liver disorder, central nervous system, digestive system, metabolic disorders and for Neuroprotective effects. Given their potential to generate significant therapeutic effect, they can be useful as drug or supplement in the treatment in the management of many diseases. Herbal drug or medicinal plants, their extract and isolated compounds have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folk-fore or food supplement for various disorders.

Boerhaavia diffusa (Punarnava) is one of the most famous medicinal plants in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. This work explains the evidence-based information regarding the pharmacological activity of this plant. It has many ethnobotanical uses (the leaves are used as vegetable; the root juice is used to cure asthma, urinary disorders, leukorrhea, rheumatism, and encephalitis). The *Boerhaavia diffusa* plant contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and

glycoproteins. Punarnavine, boeravinone, hypoxanthine 9-L-arabinofuranoside, ursolic acid, punarnavoside etc.

Now this investigation was undertaken to study the effect of *Boerhaavia diffusa* on MPTP induced Neurodegenaration and also planned to study the changes occuring in the enzymic and non enzymic system such as super oxide dismutase, catalyase, glutathione peroxide and lipid peroxidation level present in the living body.

PLAN OF WORK

- Induction of Parkinson's syndrome in rats using MPTP injection by Intra peritoneally.

- Evaluation of locomotor activity for the efficacy of drugs to decrease rigidity.

- Evaluation of efficacy of drugs using elevated plus maze test to improve cognitive function.

- Biochemical parameters include:
  - Lipid hydroperoxides
  - Catalase
  - Superoxide dismutase
  - Glutathione peroxidase
  - Total antioxidants

- Histological examination of the midbrain region to evaluate the efficacy of drugs to decrease MPTP deterioration.

Dr. D. Stephen,
Lecturer
Department of Botany

The American College,
Madurai-2

CERTIFICATE

This is to certify that the plant specimen brought to me by **Mr.NIRUBAN CHAKKARAVARTHI.G,** Second year **M. Pharm (Pharmacology); Student of K. M. College of pharmacy,** Madurai has been identified as ***Boerhaavia diffusa linn*** Belonging to the family **Nyctaginaceae.**

Dr.D.Stephen.

Date ; 06/06/2014
Madurai
Tamil nadu

THE AMERICAN COLLEGE MADURAI DEPARTMENT OF BOTANY

PLANT PROFILE

Fig.No.14

***Boerhaavia diffusa* linn**

Fig.No.15

Boerhaavia diffusa linn

PLANT PROFILE

Scientific Name : *Boerhaavia diffusa*linn. Syn. *B. repens; B. repens* var.*diffusa.*

Family : Nyctaginaceae.

Family Name : Hog weed, Horse Purslane.

TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae.

Family : Nyctaginaceae.

Division : Magnoliophyta.

Class : Magnoliopsida.

Order : Caryophyllales.

Genus : *Boerhaavia*.

Species : B.diffusa.

COMMON NAMES:

Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika.

VERNACULAR NAMES:

Bengali : Raktapunarnava.

English : Horse Purslene, Hog Weed.

Hindi : Gadapurna, Lalpunarnava.

Kannada : Sanadika, Kommeberu, Komma.

Malayalam : ChuvannaTazhutawa.

Tamil : Mukurattai (Shihappu).

Telugu : Atikamamidi, Erragalijeru.

GEOGRAPHICAL DISTRIBUTION:

*Boerhaavia diffusa*is also indigenous to India; it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. The genus *Boerhaavia* has several species, and is distributed in the tropical, subtropical, and temperate regions of the world. It is found in Australia, China, Pakistan, Sudan, Sri Lanka, Egypt, South Africa, USA and in several countries of the Middle East. Out of the 40 species of this genus, 6 species are found in India – *B. diffusa, B. chinensis, B. erecta, B. repens, B.rependa*, and *B. rubicund.* [(92)]

ORIGIN AND HABITAT:

*Boerhaavia diffusa*is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, havingthis is found throughout India. It grows up to an altitude of 70 centimeters especially during the rainy season. It has a large root system and produces yellow and white flowers. It can be found in many tropical and warm-climate countries. [(93)]

USEFUL PARTS:

Root, leaves & seeds, stem, flowers, fruits.

DESCRIPTION:

*Boerhaavia diffusa*is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having spreading branches.

The roots are very variable diffusely branched low spreading or creeping herbaceous perennial with an elongated fusiform or tapering tap root. The roots are stout and fusiform with a woody.

The stems are numerous; 1-2 m long &the stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at the nodes.

Leaves are simple, thick, fleshy and hairy arranged in unequal pairs, green and glabrous. The shape of the leaves varies considerably ovate oblong, round or subcordate at the base and smooth above. The leaves are simple, opposite, short petiolate, exstipulate, unequal in each pair, 2.5-5 cm long by 1-4.5 cm wide, oblong or suborbicular, acute, obtuse or rounded at apex, cordate rounded or truncate at base, entire or wavy along the margin, subfleshy, glabrous or sparingly hairy above, silvery white beneath, petioles 0.7-3 cm long, slender, deeply grooved above.[94]

The flowers are small, regular, sessile or subsessile, pale rose to pink, in irregular clusters of 4-10, small umbels on extra axillary peduncles. [95]

The fruits are very small, one seeded and enclosed in persistent lower half the perianth. The perianth is covered with sticky glandular hairs. [96]

Part	*Boerhaaviadiffusa*
Plant	A perennial herb from a fusiform root
Leaves	Opposite or sub-opposite, two of a node unequal, broadly ovate or sub-orbicular, obtuse to rounded or sub-cordate at the base.
Stem	Prostrate, decumbent or ascending, 4-10 cm long, rather slender, divaricately branched
Flowers	In pendunculate, glomerulate clusters arranged in slender, long stalked, axillary or terminal corymbs
Fruit	Ovoid or sub-ellipsoid, rounded above, slightly cuneate, below, broadly and bluntly 5-ribbed, very glandular throughout
Flowering and Fruiting	Throughout the year in Indian conditions

Table No.3

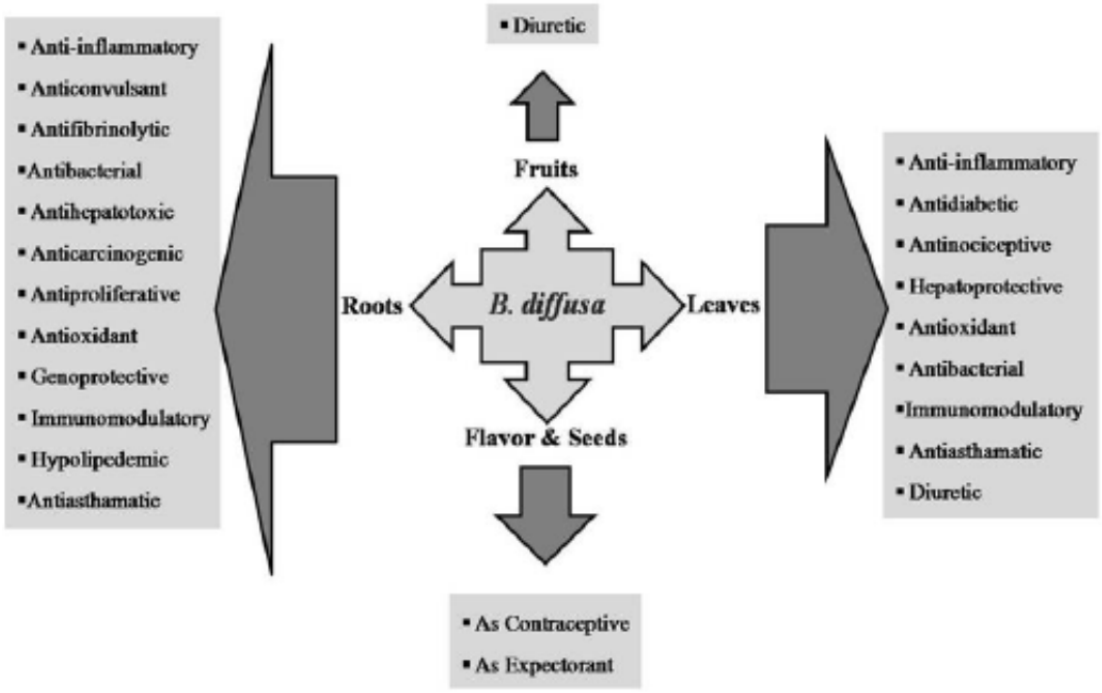
PHYTOCHEMICALS:

Boerhaavia diffusa contains a large number of phytoconstituents, namely flavonoids , alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates proteins and glycoproteins. [97]

- Plant also includes a series of rotenoids boeravinones from roots of the plant viz., Boeravinone (A-F)
- Punarnavoside, a phenolic glycoside, is reportedly present in roots C-methyl flavone also has been isolated from *Boerhaavia diffusa*roots .[98,99]
- Two known lignansviz.,liriodendrin and syringaresinol mono-β-D-glycoside isolated.[100,101]
- Presence of a purine nucleoside hypoxanthine 9-L-arabinose, dihydroisofuroxanthone-borhavine , phytosterols have been isolated from the plant.[102]
- It contains about 0.04 % of alkaloids known as punarnavine and punernavoside an anti fibrinolytic agent.[103]
- It also contains about 6 % of potassium nitrate an oily substance and ursolic acid.
- The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids.
- The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid.[104]

PHARMACOLOGICAL AND CLINICAL PROPERTIES OF *B. DIFFUSA*

Various parts of *B. diffusa* are used for the treatment of numerous disorders in different parts of India. The root, leaves, aerial parts or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine. The pharmacological studies have demonstrated that the roots of *B. diffusa* exhibit a wide range of properties such as hepato protectant.[105,106] anticonvulsant activity, immunomodulatory activity, It is clinically proved as a useful and safe drug in the patient of nephritic syndrome & cancer chemo preventive property against papillomagenesis. Potent antibacterial activity, diuretic, anti-inflammatory, antifibrinolytic, antibacterial, antistress agent, antihepatotoxic[107] anthelmintic febrifuge, antileprosy, anti-asthmatic, antiscabies, and anti-urethritis, and anti nematodal activity .[108,109]



pharmacological and clinical properties of *Boerhaavia diffusa*

Fig.No.16

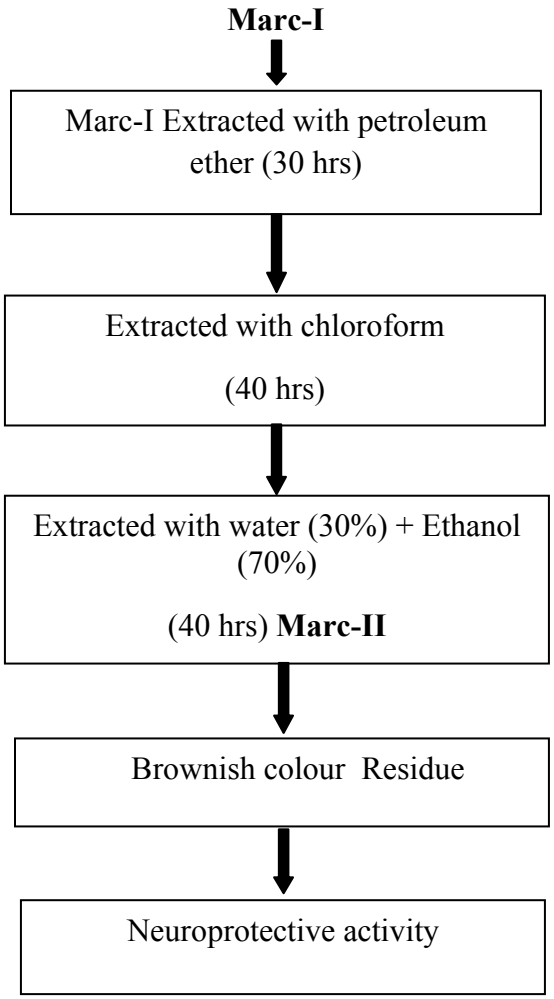
PHYTOCHEMICAL AND QUALITATIVE ANALYSIS

EXTRACTION METHODS:

Whole plant of *Boerhaavia diffusa* were collected from Tamilnadu, kerala forest, shed dried for a week in a shadow and blended to coarse powder.

About 500gm of dried fine powder of *Boerhaavia diffusa* were soaked in the extractor and macerated for 30 hrs with petroleum ether .There it is reflexed successfully with chloroform,after that it is extracted with alcohol and water by continuous hot percolation method using soxhlet apparatus for 40hrs separately. Hydro alcoholic extracted was filtered and concentrated in vacuum using rotary flask evaporator under reduced pressure .After concentration hydro alcoholic extract of *Boerhaavia diffusa* given brownish residue stored in air tight container were subjected to qualitative test for identification of various plant constituents.

Dried whole plant of *Boerhaavia diffusa*material extracted by soxhletmethod



Extraction procedure.

PHYTOCHEMICAL INVESTIGATION OF EXTRACTS OF *Boerhaavia diffusaLinn.*

All the extracts of leaf of *Boerhaavia diffusa* linn were subjected to various tests for identification of constituents.

1) Detection of Carbohydrates:

Small quantities of ethanolic and aqueous extract were dissolved in distilled water separately and filtered. The filtrates were taken for the various tests to detect the absence of carbohydrates. Absence of carbohydrate in *Boerhaavia diffusa.*

A. Molisch's Test:

The filtrates were treated with 2-3 drops of 1% alcoholic α- napthol and 2 ml. of concentrated sulphuric acid was added along the sides of the test tube. A brown ring was observed. Ethanolic *Boerhaavia diffusa* plant extract showed the absence of sugar.

B. Fehling's Test:

Small portion of the filtrates were treated with equal volume of Fehling's solution A and B and then heated. A brick red precipitate formed in alcoholic plant extract of *Boerhaavia diffusa* show absence of reducing sugar.

C. Benedict's Test:

Small portion of the filtrates were treated with equal volume of Benedict's reagent. A yellow precipitate was formed in alcoholic extract *Boerhaavia diffusa* indicating the absence of reducing sugar

D. Barfoed's Test:

Small portion of the both the plant extract was treated with Barfoed's reagent. Red precipitate was not formed in the ethanolic plant extract *Boerhaavia diffusa*.

E. Test for Starch:

A small amount of the *Boerhaavia diffusa* ethanolic plant extract was treated with dilute iodine solution. No bluish black colour was observed in the both the plant extracts showing the absence of starch**.**

2) Tests for Gums and Mucilage's:

Alcoholic precipitation and Molisch's test.

The, *Boerhaavia diffusa* plant extract was treated with absolute alcohol, stirred and filtered. The filtrate was dried and examined for its swelling properties. The extracts were answered for the presence of gums and mucilage.

3) Test for Proteins and Amino Acids:

Small quantities of alcoholic extract was dissolved in few ml of distilled water and subjected to Ninhydrin test, Xanthoprotein test, test with tannic acid and heavy metals.

A. Ninhydrin Test:

Ethanolic extract of the both the plants were treated with ninhydrin reagent (0.1% solution) and boiled. Purple colour was observed indicating the presence of protein.

B. Biuret Test:

To a portion of the above prepared extracts, equal volumes of 5% w/v sodium hydroxide and 4 drops of 1% w/v copper sulphate solution were added. Violet colour was formed, indicating the presence of protein in the extract.

C. Millon's test of cole's mercuric nitrite test:

To the above-prepared extracts, millon's reagent was added. White precipitate was formed, showing the presence of protein in the extract.

D. Xanthoprotein Test:

To 3 ml of the above-prepared extracts, 1 ml of the concentrated nitric acid was added, boiled for one minute, cooled and concentrated ammonia was added till alkaline. An orange colour was not formed, showing the presence of protein in the extracts.

4) Test for Fixed Oils and Fats:

A. Spot Test:

A small quantity of various extracts was pressed between two filter papers. Oil stains were observed with the extracts indicating the presence of fixed oils and fats.

B. Saponification Test:

Few drops of 0.5 N alcoholic potassium hydroxide was added various extracts along with of few drops of phenolphthalein. The mixture was heated on a water bath for one hour. Soap was formed with the extracts indicating the presence of fixed oils and fats.

5) Test for Alkaloids:

Small amount of the solvent free ethanolic and aqueous extracts were separately stirred with a few ml of dilute HCl and filtered. The filtrates were tested with various alcoholic reagents.

A. Mayer's test :

To the small quantities of the extracts, Mayer's reagent was added. Presence of cream-colored precipitate indicates the presence of alkaloids in both the extracts.

B. Dragendorff's Test:

To small quantity of extracts, Dragendorff's reagent was added. Presence of orange brown precipitate indicates the presence of alkaloids in both plant extracts.

C. Wagner's Test:

To small quantity of the extracts, Wagner's reagent was added. Presence of reddish brown precipitate, indicate the presence of alkaloids in *Boerhaavia diffusa* extract

D. Hager's Test:

To small quantity of the extracts, Hager's reagent was added. Presence of yellow precipitate, indicate the presence of alkaloids in *Boerhaavia diffusa* extract.

6) Tests for Glycosides:

A small amount of the different extracts were dissolved separately in 5 ml of distilled water and filtered. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolyzate was subjected to Legal's, Baljet's, Borntrager's, Keller-Killani's tests and for the presence of cyanogenetic glycosides.

A. Legal's Test:

To the hydrolyzate, 1 ml of pyridine and a few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Pink colour was observed in *Boerhaavia diffusa* extract.

B. Baljet's Test:

To a section of plant extract, sodium picrate solution was added. Yellowish orange colour was observed in *Boerhaavia diffusa* extract.

C. Borntrager's Test:

Hydrolyzed was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. Pink colour was not observed in the ammonical layer of chloroform and both the extracts showed the absence of glycosides.

D. Test for Deoxy Sugar (Keller-Killani Test):

To the different extracts 10 ml of 70% alcohol were added, boiled on a water bath, filtered. The filtrates were diluted with 1 ml of distilled water; 1ml of strong lead acetate solution was added and filtered. The filtrates were extracted with an equal volume

of chloroform. The chloroform layer was pipetted out and evaporated to dryness. The residue obtained was dissolved in 3 ml of 3.5% of ferric chloride in glacial acetic acid, left for one minute and then transferred to a test tube. To the side of the test tube, 1.5 ml of sulphuric acid was added carefully, which formed a separate layer at the bottom and kept for few minutes.

Blue colour at the interface and pale green colour in the upper layer was not observed in any of the extracts indicating the absence of cardiac glycoside.

7) Test for Phytosterols:

Small quantities of the various extract were dissolved in the 5 ml of chloroform separately. Then these chloroform solutions were subjected to Libermann's test, Libermann-Burchard's test, Salkowski's test.

A. Libermann-Burchard's Test:

The residue was dissolved in chloroform. To this Libermann-Burchard's reagent was added. Green colour was produced in both the extract indicating the presence of phytosterols.

B. Salkowski's test:

A few drops of concentrated sulphuric acid were added to chloroform solution. The lower layer of the solution turned brownish red colour with both the extracts indicating the presence of phytosterols.

8) Test for Flavanoids:

The different extracts were separately dissolved in ethanol and then subjected to the following tests.

A. Ferric chloride Tests:

To a small quantity of the ethanolic extract, few drops of neutral ferric chloride were added. Blackish red colour was observed in *Boerhaavia diffusa* extract indicating the presence of flavonoids.

B. Shinoda's test:

A small quantity of the extract was dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid, was added drop wise and heated. A magenta colour was produced in *Boerhaavia diffusa* extract indicating the presence of flavonoids.

C. Flavones:

1. With sodium hydroxide solution, the extract gave yellow colour.
2. Ethanolic extract gave orange colour with concentrated sulphuric acid.

9) Test For Tannins:

The extracts were dissolved in water and filtered. The filtrates were treated with various reagents.

A. Ferric chloride test:

Few ml of the filtrates were treated with 5% ferric chloride solution. A bluish black colour was observed indicating the presence of tannins in both the extracts.

B. Reaction with lead acetate:

Few ml of the filtrates were treated with lead acetate solution. White precipitates were produced in *Boerhaavia diffusa* extract indicating the presence of tannins.

C. Gelatin Test:

The extract were dissolved separately in minimum amount of water and filtered. To the filtrate, add 1 ml of 1 %solution of gelatin. Both the extract did not produce any white precipitate.

TABLE .NO:4

QUALITATIVE CHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF

Boerhaavia diffusaLinn.

SI.NO	**Test for plant constituents**	***Boerhaavia diffusaLinn.***
1	**Test for alkaloids**	
	a. Mayer's Test	+
	b. Dragendorff's Test	+
	c. Wagner's Test	+
	d. Hager's Test	+
2	**Test for Glycosides**	
	a. Legal's Test	+
	b. Baljet's Test	+
	c. Borntrager's Test	+
	d. Keller-Killani's Test	+
3	**Test for Flavanoids**	
	a. Ferric chloride Test	+
	b. Shinoda's Test	+
	c. Fluorescence Test	+

	d. Reaction with alkali and acid	+
4	**Test for Tannins and Phenolic Compounds**	
	a. 5% Ferric chloride solution test	+
	b. Reaction with lead acetate	+
	c. Gelatin test	–
5	**Test for Proteins and amino acids**	
	a. Ninhydrin Test	+
	b. Biuret Test	+
	c. Millon's test or Cole's Mercuric Nitrate test	+
	d. Xanthoprotein test	+
6	**Test for Carbohydrates**	
	a. Molisch's test	-
	b. Fehling's test	-
	c. Benedict's test	-
	d. Barfoed's test	-
	e. Test for Starch	-
7	**Test for Gums and Mucilage**	
	Alcoholic precipitation and Molisch's test	+

PHARMACOLOGICAL EVALUATION

In today's life of stress and strain the need for agents having neuroprotective and neuropharmacological activity increasing for enhancing learning and memory function of the brain.[110] Stress involves complex biochemical, neural and immunological mechanism and play a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric diseases cardiovascular diseases, hypertension, peptic ulcers, migraine, allergies, asthma, carcinoma, rheumatic diseases and ulcerative colitis.[111] The ancient system of medicine (Ayurveda) is a treasure of medicinal plants as herbal remedies, to prevent or treat diseases. The plant *Boerhaavia diffusa* Linn reported to possess strong neuroprotective effects.

MATERIALS AND METHODS

MATERIALS

1. 1- methyl -4 phenyl-1,2,3,6 –tetrahydropyridine(MPTP- neurotoxin from Sigma Aldrich, Saint Louis,MO, USA).
2. Ketamine from Neon Pharmaceuticals.
3. *Boerhaavia diffusa* Linn were collected from Tamilnadu, kerala forest, shed dried for a week in a shadow and blended to coarse powder.

ANIMALS

- Male Wistar rats weighing 200-250 gm.
- The rats were maintained in a controlled room temperature (25 ± 2^0C) on 12 hr light–dark cycles (lights on 7.00 am) with free access to food and water *ad libitum* in central animal house at K.M. College of Pharmacy animal house.
- The studies were conducted accordance with the Ethical Committee and all the animals were sacrificed by euthanasia method.
- The rats were placed in poly propylene cages with three animals per cage and were allowed to acclimatize one week prior to treatments.

METHODS

- MPTP was purchased from Sigma Aldrich, Saint Louis, MO, USA.
- The standardized Hydroalcoholic extract of *Boerhaavia diffusa* Linn bought from Tamilnadu, kerala forest, shed dried for a week in a shadow and blended to coarse powder.
- MPTP (20mg/kg) was dissolved in normal saline solution and administered through I.P route.
- Hydroalcoholic extract of *Boerhaavia diffusa* linn extract is dissolved in 0.9% saline orally for 21 days.
- Both MPTP and Hydroalcoholic extract of *Boerhaavia diffusa* Linn extract continued for 21 days.

TREATMENT PROTOCOL

The rats were segregated into five groups. Each group have six rats.

GROUP I : Served as Normal control and received normal saline (10ml/kg, orally).

GROUP II : Served as Perse control received 250mg/kg body weight of Hydroalcoholic Extract of *Boerhaavia diffusa* Linn orally for period of 21 days.

GROUP III : Served as toxic control received 20mg/kg body weight of MPTP administered through intraperitoneally.

GROUP IV : Served as treatment control received 20mg/kg body weight of MPTP administered through intraperitoneally and 200mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn orally administered for 21 days.

GROUP V : Served as treatment control received 20mg/kg body weight of MPTP administered through intraperitoneally and 400mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn orally administered for 21 days.

BEHAVIOURAL STUDIES

All behavioural studies were started 2 weeks after MPTP treatment.[112] The behavioural studies was performed between 10 .00am -2.00 pm in a laboratory at a standard optimal conditions. All experiments were performed and analysed by a subject blind to experiment.

ELEVATED PLUS MAZE TEST:

PROCEDURE:

The elevated plus maze consisted of two opposite black open arms (50 × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10 × 10 cm. The entire maze was elevated to a height of 50 cm from the floor. Acquisition of memory was tested on day 13 after MPTP administration. Animal was placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as the initial transfer latency (ITL). Animal was allowed to explore the maze for 20 seconds after recording the ITL and then returned to the home cage. If the animal did not enter the enclosed arm within 90 seconds, it was guided on the back into one of the enclosed arm and the ITL was given as 90 seconds. Retention of memory was assessed by placing the rat in an open arm and the retention latency was noted on day 14 and day 21 of ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.[113]

TREATMENT PROTOCOL:

G1: Served as Normal control and received normal saline (10ml/kg, orally).

G2: Served as Perse control received 250mg/kg body weight of Hydroalcoholic Extract of *Boerhaavia diffusa* Linn orally for period of 21 days.

G3: Served as toxic control received 20mg/kg body weight of MPTP administered through intraperitoneally.

G4: Served as treatment control received 20mg/kg body weight of MPTP, intraperitoneally and 200mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn 200mg/kg orally and administered for 21 days.

G5: Served as treatment control received 20mg/kg body weight of MPTP, Intraperitoneally and 400mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn 400 mg/kg orally administered for 21 days.

LOCOMOTOR ACTIVITY

The locomotor activity was measured using an Actophotometer. The movement of the animal cut off a beam of light falling on photocell and count was recorded and displaced digitally.

PROCEDURE

Each rat was placed individually in the actophotometer for 10 minutes and basal activity scores were recorded .Gross behavior activity was observed on 14th and 21st day after MPTP injection .The animals were observed for a period of 10 minutes and the values were expressed as counts/10min.**(114)**

TREATMENT PROTOCOL:

G1: Served as Normal control and received normal saline (10ml/kg, orally).

G2: Served as Perse control received 250mg/kg body weight of Hydroalcoholic Extract of *Boerhaavia diffusa* Linn orally for period of 21 days.

G3: Served as Toxic control received 20mg/kg body weight of MPTP intraperitoneally.

G4: Served as Treatment control received 20mg/kg body weight of MPTP intraperitoneally and 200mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn orally and administered for 21 days.

G5: Served as Treatment control received 20mg/kg body weight of MPTP intraperitoneally and400 mg/kg body weight of Hydroalcoholic extract of *Boerhaavia diffusa* Linn orally administered for 21 days.

BIOCHEMICAL EVALUATIONS

On completion of experimental period, animals are sacrificed by euthanasia under ketamine Anaesthesia. Brain tissues are excised immediately and immersed in ice cold saline. The tissues were homogenized using homogenizer. The homogenate was centrifuged at 12000 rpm for 20 minutes, 40°C to obtain the post mitochondrial supernatant (PMS) which was used for further analysis.

ESTIMATION OF GLUTATHIONE PEROXIDASE

Glutathione peroxidase is an enzyme found in cytoplasmic and mitochondrial fraction of cells which catalyse the reduction of hydrogen peroxide by reduced glutathione and function to protect the cells from oxidative damage. The determination of Glutathione peroxidase using ELISA kits measures GP_x indirectly by a coupled reduction with glutathione reductase. Oxidised Glutathione (GSSG) produced upon reduction of hydroperoxide by GP_x is recycled to its reduced state by GR and NADPH.[115]

$$ROOH + 2GSH \xrightarrow{GP_X} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP+$$

The oxidation of NADPH to NADP+ is monitored spectrophotometrically by a decrease in Absorbance at 340 nm (A340). Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity in the sample. By means of these kits we can determine all of the glutathione dependent peroxides in plasma, erythrocytes, lysates and tissues

ESTIMATION OF SUPEROXIDE DISMUTASE

Superoxide dismutase is metalloenzymes that catalyse the dismutation of superoxide into hydrogen peroxide and molecular oxygen and provide an important defense mechanism against superoxide radical toxicity.

A common indirect method for SOD utilizes nitroblue tetrazolium conversion to NB+ diformazan (formazan dye) via superoxide radical.The rate of reduction with

O2- is related to XO activity and inhibited by SOD.IC50 can be detected by colourimetry by using absorbance at 440nm.[116]

ESTIMATION OF TOTAL ANTIOXIDANTS

Reactive oxygen species may be obtained as side products of mitochondrial oxidative phosphorylation utilized by brain. Excess production of reactive oxygen species causes oxidative stress. The antioxidant systems include enzymes such as Superoxide dismutase, Catalase, glutathione peroxidase, macromolecules such as albumin, ceruloplasmin, ferritin and array of small molecules such as Ascorbic acid,α-Tocopherol, β-carotene. The sum of the endogenous and food derived antioxidants represent total antioxidant capacity of the system.

ELISA kits can be used to measure the total antioxidant capacity of serum, urine, saliva or cell lysates.

The principle involved in this assay is ability of antioxidants in the sample to inhibit the oxidation of ABTS (2-2' azino-di [3 ethyl benzthiazoline sulphonate] to ABTS + by metmyoglobin. The amount of ABTS+ produced can be monitored by reading the absorbance at 750nm or 405 nm.[117] Under the reaction condition used the antioxidants of the sample cause suppression of absorbance at 750nm or 405 nm to a degree which is proportional to concentration.

ESTIMATION OF CATALASE

Catalase is a ubiquitous antioxidant enzyme which is involved in the detoxification of hydrogen peroxide (H_20_2), a reactive oxygen species which is a toxic product of both normal aerobic metabolism and pathogenic ROS production.

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2+2H_2O$$

$$H_2O_2+AH_2 \xrightarrow{\text{Catalase}} A+2H_2O$$

This assay utilizes the peroxidative function of CAT for determination of enzyme activity, which is based upon the reaction of enzyme with methanol in the

presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino 3 hydrazino 5 mercapto-1, 2, 4 triazole.[118]

ESTIMATION OF LIPID HYDROPEROXIDES

Lipid peroxidation, a major indicator of oxidative stress measured by using thiobarbituric acid substance.[119] This method is utilized for assaying samples such as drugs, food products and human and biological tissues. It provides important information regarding free radical activity in diseases and used for measurement of antioxidant activity of several compounds. The principle involved in this assay is Malonidialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid and produces the compound which can be measured by flurimetry.

HISTOPATHOLOGICAL EXAMINATION:

Mid portion of the brain specimens obtained from all groups of animals were fixed in 10% formalin. The tissue sections were embedded in Paraffin wax and sectioned at 5-6μm thickness and sections were stained with Haematoxylin and eosin method for photomicroscopic observation of the brain histopathological architecture.[120]

RESULTS

Effect of Hydroalcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) on LPO in MPTP Treated rats

Systemic administration of MPTP causes significant rise in brain LPO (Lipid hydroperoxides) levels in MPTP treated groups (G3) compared to control groups (G1) at P<0.01.In groups treated with (HAEBD) alone there is no significant change in LPO levels (P>0.05).In groups treated with both doses of (HAEBD) (200mg and 400mg/kg) along with MPTP, there was a significant decrease (p<0.01) in LPO levels. **(Table. 5) (Chart.1)**

Effect of Hydroalcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) on SOD in MPTP treated rats

Brain homogenate tissue SOD levels were significantly decreased in MPTP treated animals in (Group 3) compared to control groups (G1) at (p<0.01).In groups (G2) animals treated with HAEBD alone there is no significant change in SOD levels.but treatment with both doses of HAEBD 200mg/kg and 400mg/kg in Group 4 &5 significantly increased the SOD levels after 21 days (p<0.01). **(Chart.2)**

Effect of Hydroalcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) on catalase in MPTP treated rats

There was statistically significant decrease in Catalase levels after MPTP treatment for 21 days (p<0.01). HAEBD alone did not change significant catalase levels(p>0.05). But HAEBD administered for 21 days along with MPTP was able to increase the CAT levels more than MPTP alone(p<0.01). (**CHART.3**)

Effect of Hydroalcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) on glutathione peroxidase (GP_X) in MPTP treated rats

HAEBD alone when administered at a dose of 250mg/kg, no significant change in GP_x levels (p>0.05). But MPTP treatment decrease the GP_X levels significantly (p<0.01) and treatment with HAEBD at both doses (200mg/kg and 400mg/kg) along with MPTP was able to increase the GP_x levels. Brain tissue homogenates GP_X level reached near normal in both groups treated with HAEBD (p<0.01). **(CHART.4)**

Effect of Hydroalcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) on total antioxidants (TA) in MPTP treated rats

There was a significant decrease in total antioxidants (TA) levels after exposure of rats to MPTP ($p<0.01$) for 21 days. Concurrent treatment with HAEBD at two doses 200mg/kg and 400mg/kg significantly increased total antioxidant levels ($p<0.01$).but in groups (G2) animals treated with HAEBD alone there is no significant change in TA levels. ($p>0.05$). (**CHART.5)**

Effect of improved on Behavioural alteration in MPTP treated rats

Elevated plus maze:

In the present experiment, mean initial transfer latencies (ITL) on day13 for each rat was relatively stable and showed no significant variation among different groups. All the rats entered the closed arm within 90 seconds Following training, normal control, MPTP injected, and Hydro alcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) treated (200mg and 400mg/kg) rats entered closed arm quickly as compared to MPTP treated rats. Mean retention transfer latencies (1^{st} RTL and 2^{nd} RTL) to enter closed arm on day 14 and 21 were shorter as compared to ITL on day 13 of each group, respectively. In Contrast, MPTP injected rats performed poorly throughout the experiment and did not show any change in the Mean retention time transfer latencies on day14 and 21 as compared to the pre training latency on day 13,demonstrating that MPTP induced marked memory impairment.

Chronic Administration of Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) (200mg&400mg/kg) beginning prior to MPTP injection significantly decreased mean retention latencies on day 14&21 following MPTP injection. ($p<0.01$).

The mean transfer latencies of Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) treated (200mg/kg&400mg/kg p.o) and MPTP treated groups were significantly different from that of HAEBD perse groups on day 14&21. ($p<0.05$). **(Table.6)**

Effect of Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) on locomotor activity

In the present series of experiments the mean scores of locomotor activity for each rat were more relatively stable and showed no significant variation among different groups. The mean scores in normal control, perse control and MPTP treated rats remain unchanged. Further, both the dose of Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) (200mg&400mg/kg) did not cause any significant activations in the locomotor activity as compared to MPTP treated rats on day 14&21. **(Table.7)**

Histological examination of the Rat's brain

- **Fig no: 17** Section of brain from rats treated with Normal Saline for 21 days showing normal architecture.
- **Fig no: 18** Section of brain from rat treated with 250mg/kg body weight of Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) for 21 days (perse control) showing normal architecture.
- **Fig no: 19** section of brain from rats treated with MPTP for 21 days showing pathological changes like cellular inflammation, vascular degeneration and cytoplasmic vacuolation.
- **Fig no:20 & 21** section of brain from rats treated with MPTP for 21 days followed by Hydroalcoholic extract of *boerhaavia diffusa* Linn (HAEBD) treatments showing marked reduction of degeneration and vacuolation.

BIOCHEMICAL PARAMETERS

Table.5:-EFFECT OF HAEBD ON ANTIOXIDANT STATUS IN HOMOGENENATE OF BRAIN TISSUES

GROUPS	Lipidhydroperoxide LPO(nmol/mg of protein)	Total antioxidants TA(mM/mg of protein)	Glutathione Peroxidase GP_x(nmol/min /mg of protein)	Catalase CAT(µM/ mg of protein)	Superoxide dismutase(units /mg of protein)
GROUP I	3.03±0.05**	3.10±0.07**	22.23±0.83**	46.45±1.15**	35.58±1.19
GROUP II	2.48±0.04	3.23±0.08	27.34±0.99	52.94±0.95	33.21±0.71
GROUP III	4.35±0.10	1.6±0.07	12.45±0.58	32.19±1.14	9.87±0.82
GROUP 1V	2.14±0.11**	2.41±0.04**	20.37 ±0.97**	45.45±0.61**	20.03±0.84
GROUP V	2.52± 0.05**	2.6±0.16	22.03±0.70**	46.76±0.77**	23.26±0.69**

Values are expressed as Mean±SEM.

Values were found out by using one way ANOVA followed by Newman Keul's multiple range tests.

- a **-Values are significantly different from Normal control at P<0.01.
- b*-Values are significantly different from Toxic control at P<0.05.
- b**-Values are significantly different from Toxic control at P<0.01.

Table.6:-EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN (HAEBD) ON MEMORY PERFORMANCE IN ELEVATED PLUS MAZE PARADIGM IN MPTP INJECTED RATS.

GROUPS	TREATMENT	**MEAN TRANSFER LATENCY**		
		ITL	**ISTRTL**	**2nd RTL**
GROUP I	Normal control (10ml/kg Normal saline)	60.48±0.483	21.61±0.31**	22.58±0.62**
GROUP II	Perse control (Extract alone-250mg/kg body weight HAEBD)	63.47±0.75	20.06±0.44	18.44±0.58
GROUP III	Toxic control (20mg/kg body weight of MPTP)	68.61±0.59	82.03±1.34	80.57±1.20
GROUP 1V	Treatment control (20mg/kg body weight of MPTP+200mg/kg body weight of HAEBD)	64.84±0.46**	48.32±1.42**	41.90±1.10
GROUP V	Treatment control(20mg/kg body weight MPTP +400mg/kg body weight of HAEBD)	66.21±0.55*	38.40±0.93*	35.27±0.80**

Values are expressed as Mean± SEM

Values were found out by using one way ANOVA followed by Newman Keul's multiple range tests.

*a-Values were significantly different from Normal and Perse Control at $p<0.01$.

*b -Values were significantly different from MPTP treated groups at $p<0.05$.

TABLE .7:- EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN (HAEBD) ON LOCOMOTOR ACTIVITY

GROUPS	TREATMENT	Locomotor activity (score) in 10 min±SEM on	
		14th day	21st day
GROUP I	Normal Control	216.06±1.61*	222.18±1.46**
GROUP II	Perse Control	203.84±1.92	216.13±1.76
GROUP III	Toxic Control	221.49±1.69	236.60±1.60
GROUP IV	Treatment control	200.39±1.94**	226.57±1.86*
GROUP V	Treatment control	231.75±1.08**	229.42±2.01*

Values are expressed as Mean±SEM

Values were found out by one way ANOVA followed by Newmann Kaul's Multiple range test

Values were not significantly different from each group.

EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN (HAEBD) ON LIPID HYDROPEROXIDES

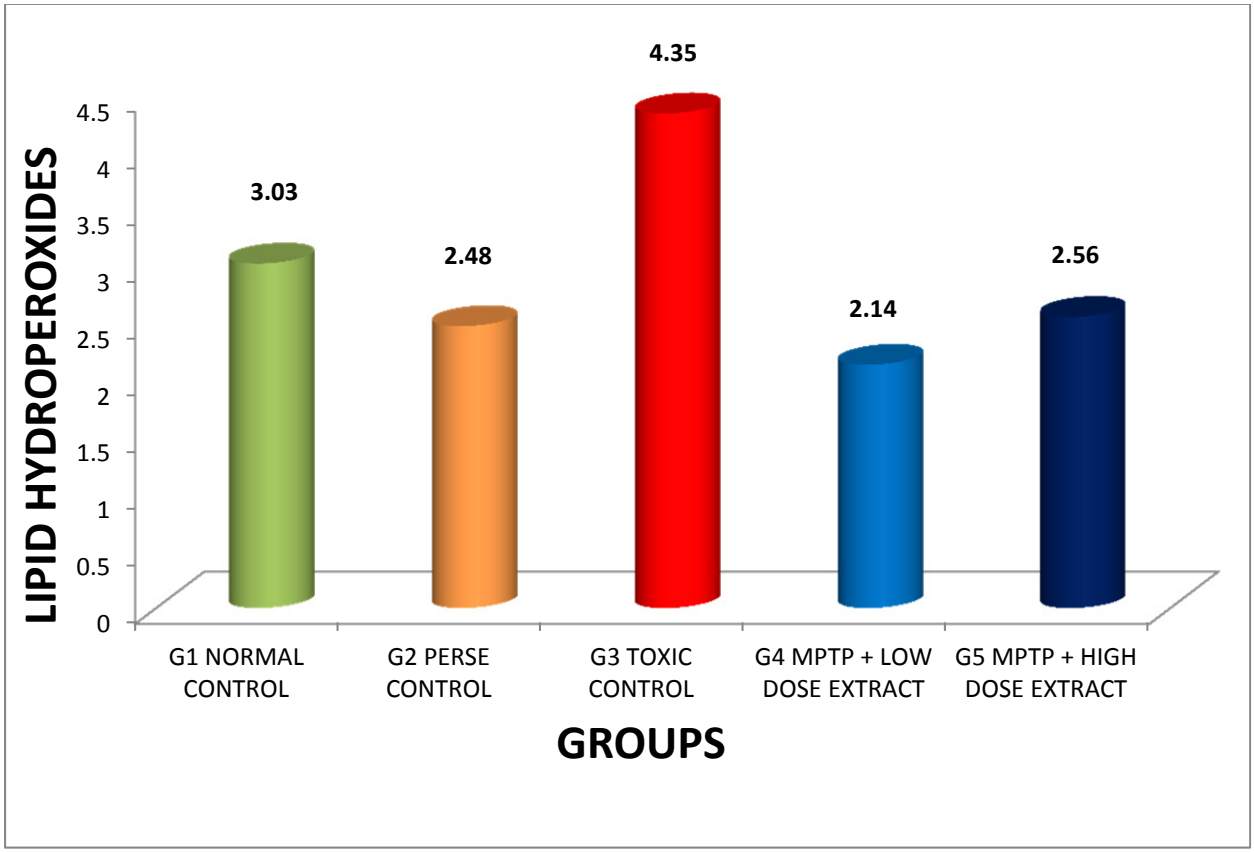


CHART1

EFFECT OF HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD)

SUPEROXIDE DISMUTASE

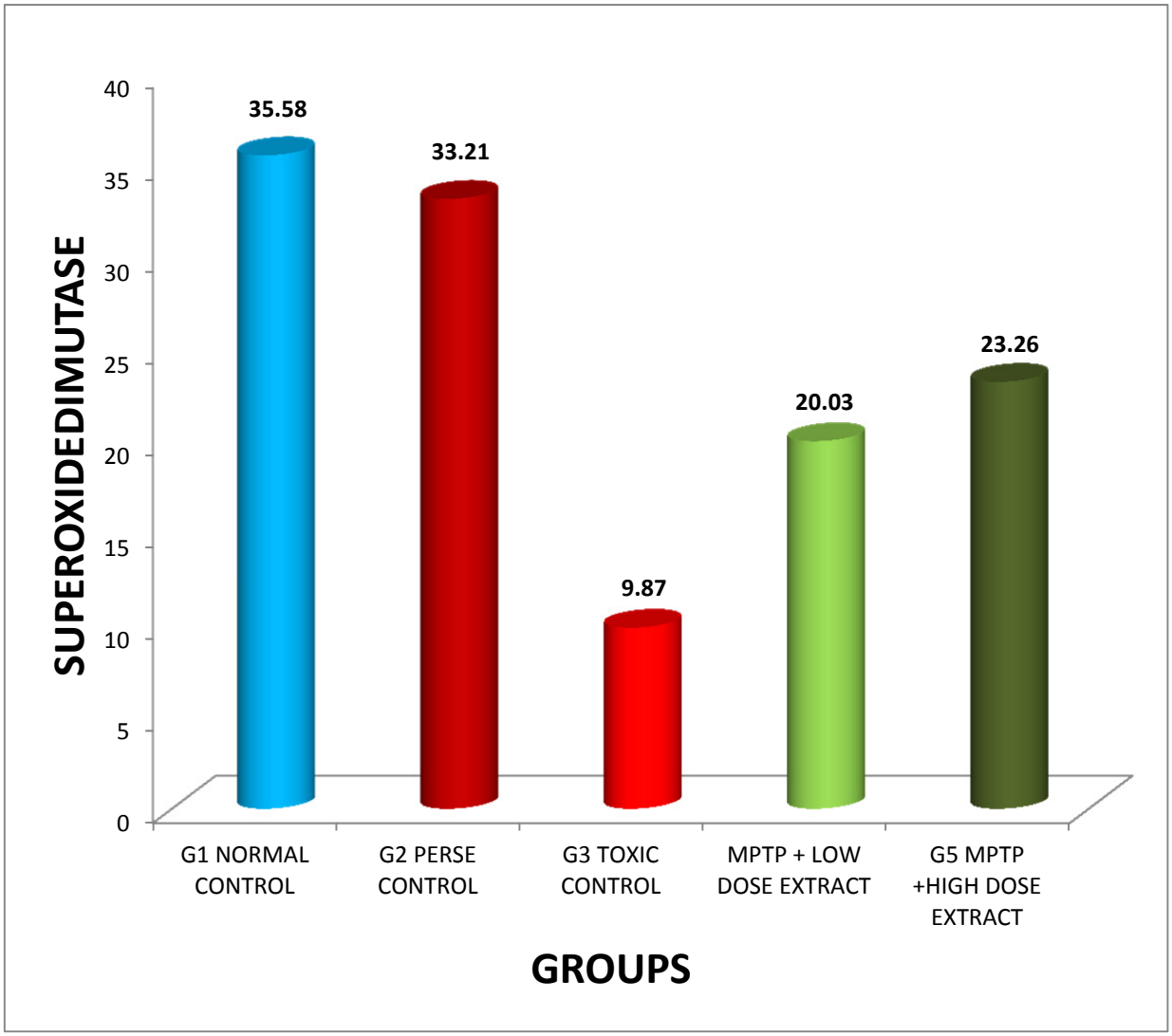


CHART 2

EFFECT OF HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD)

ON CATALASE

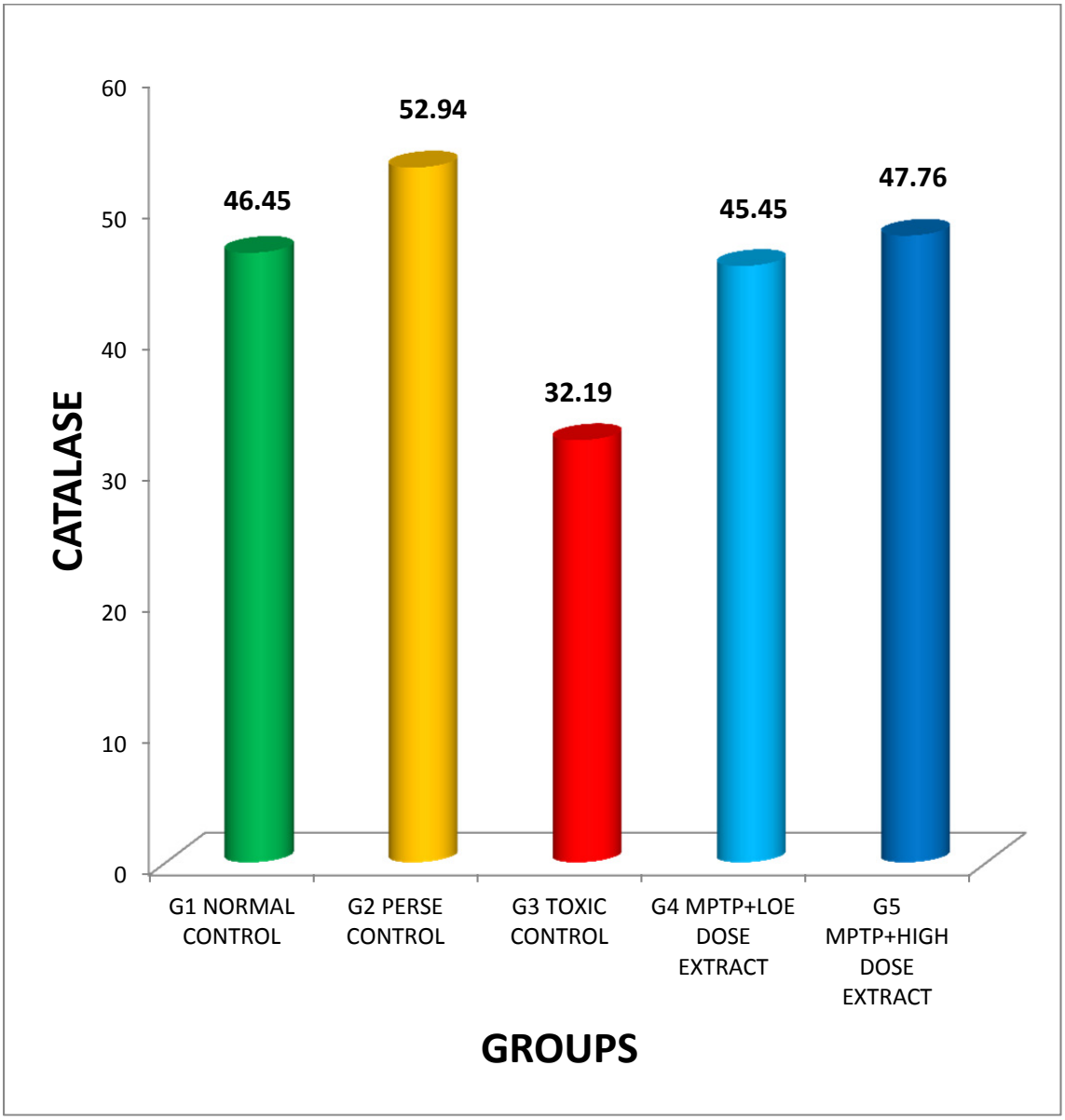


CHART 3

EFFECT OF HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD)

ON GLUTATHIONE PEROXIDASE

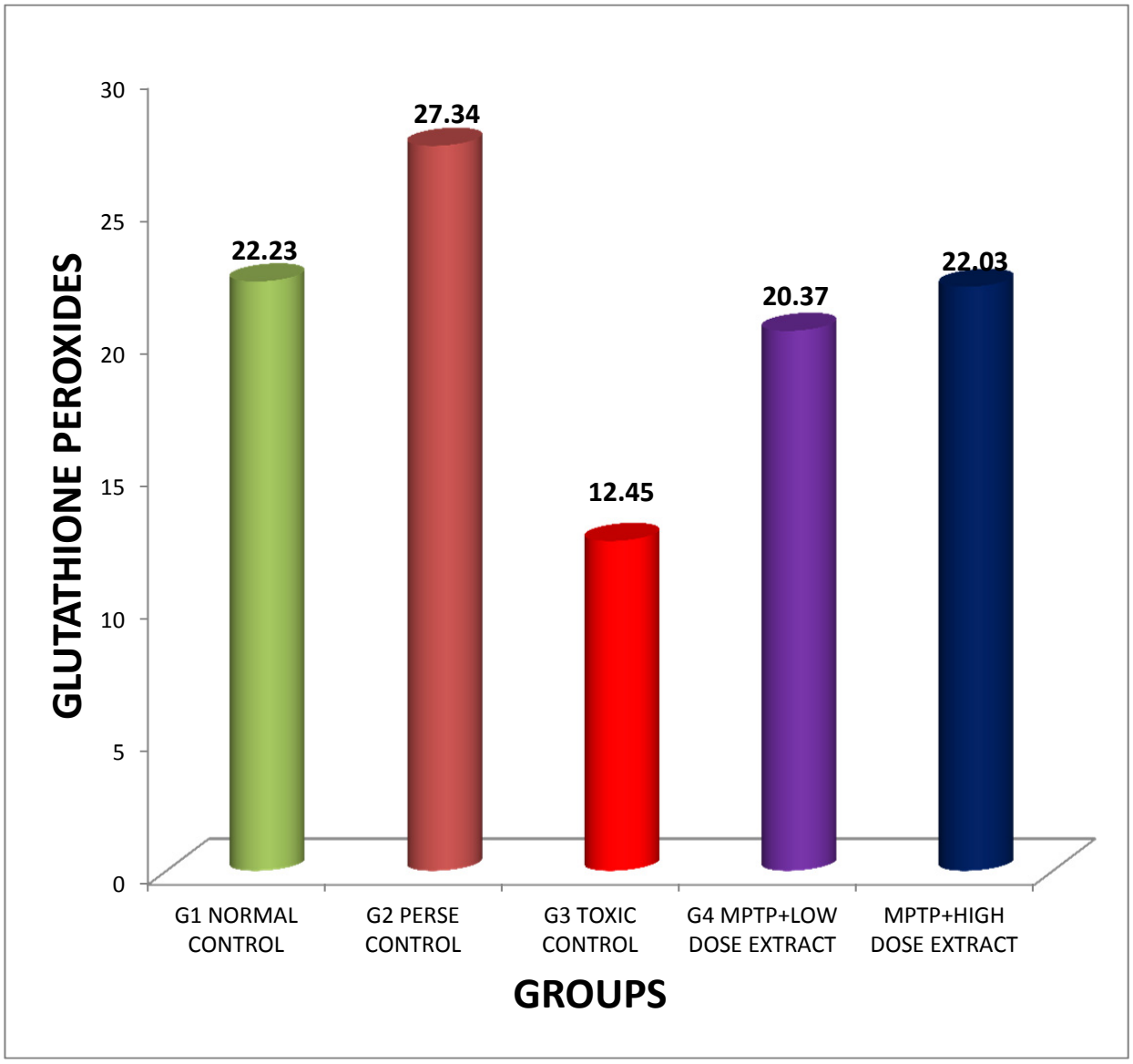


CHART 4

EFFECT OF HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD)

TOTAL ANTIOXIDANTS

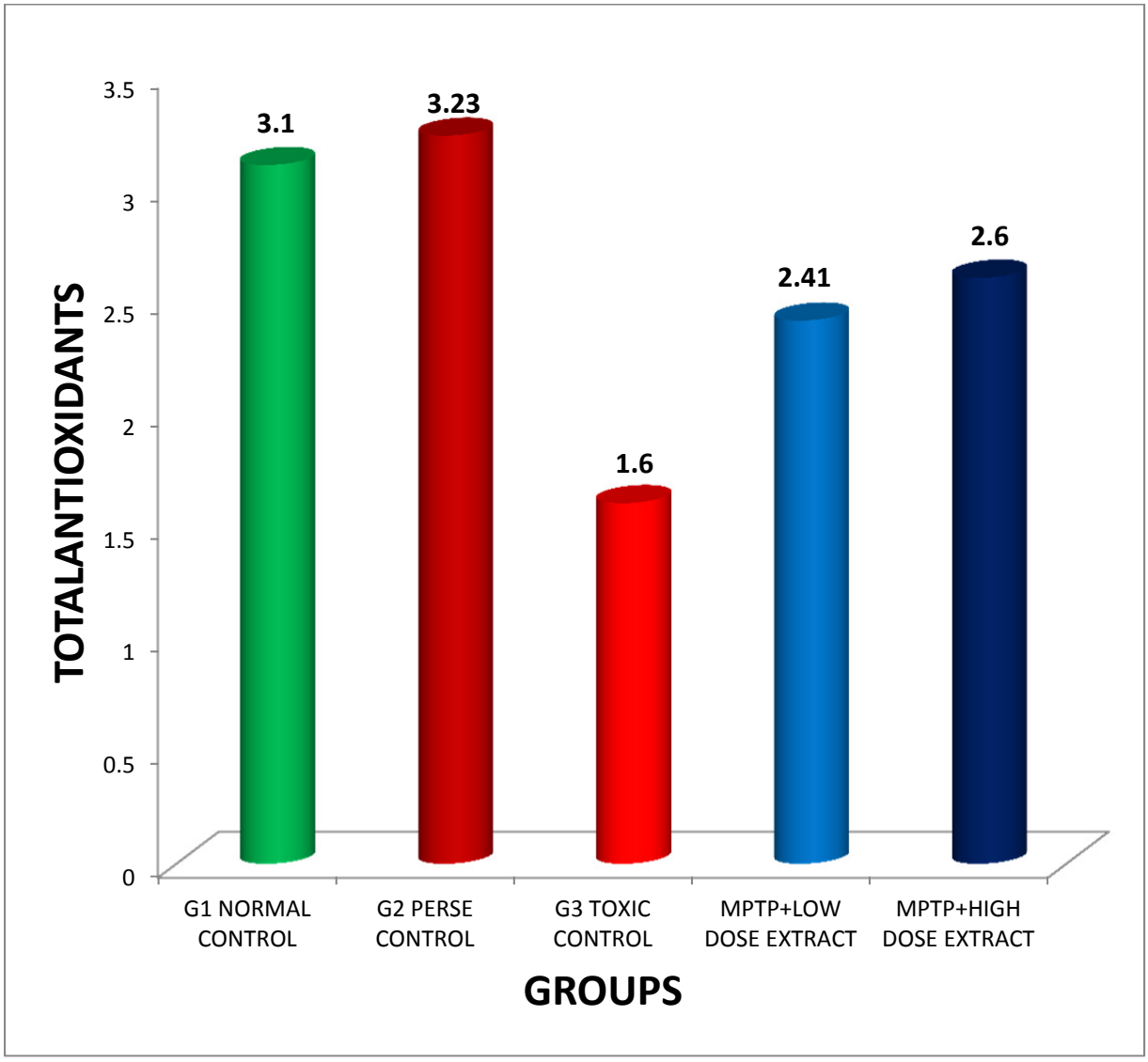


CHART 5

HISTOPATHOLOGICAL EXAMINATION

G1: NORMAL CONTROL (10ml/kg Normal saline)

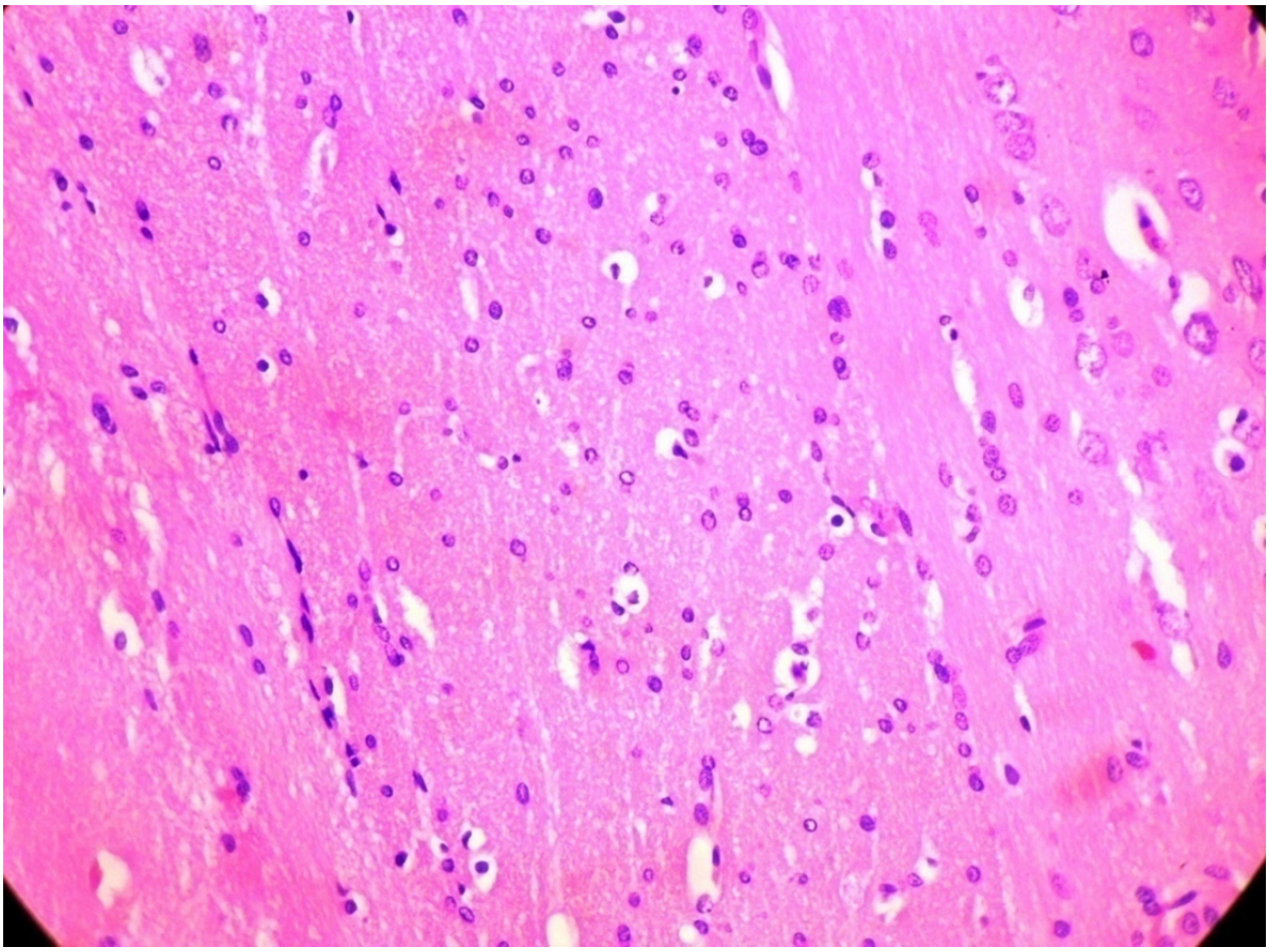


FIG NO:17

G2: PERSE CONTROL (Extract alone-250mg/kg)

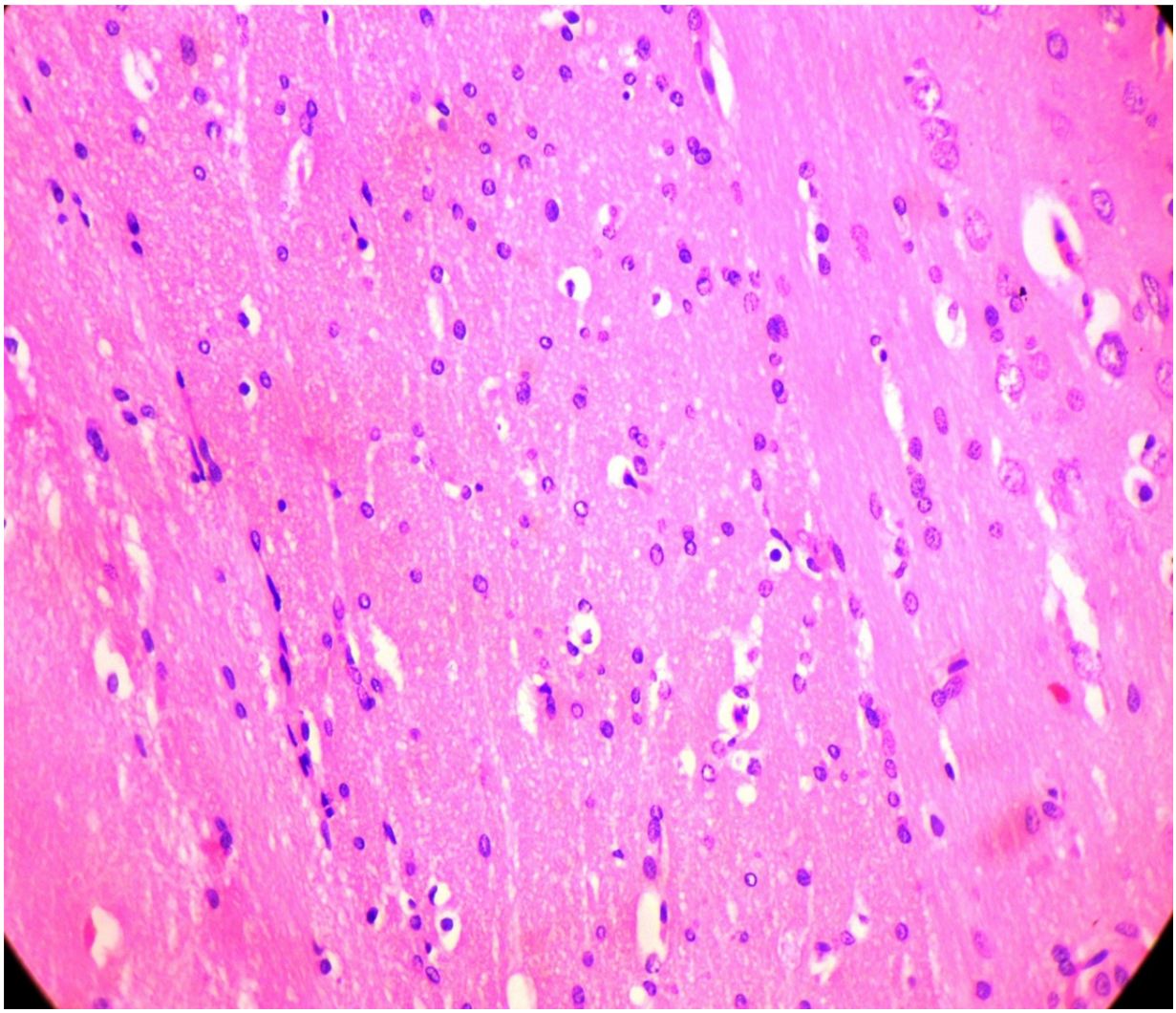


FIG NO:18

G3: TOXIC CONTROL (MPTP alone-20mg/kg)

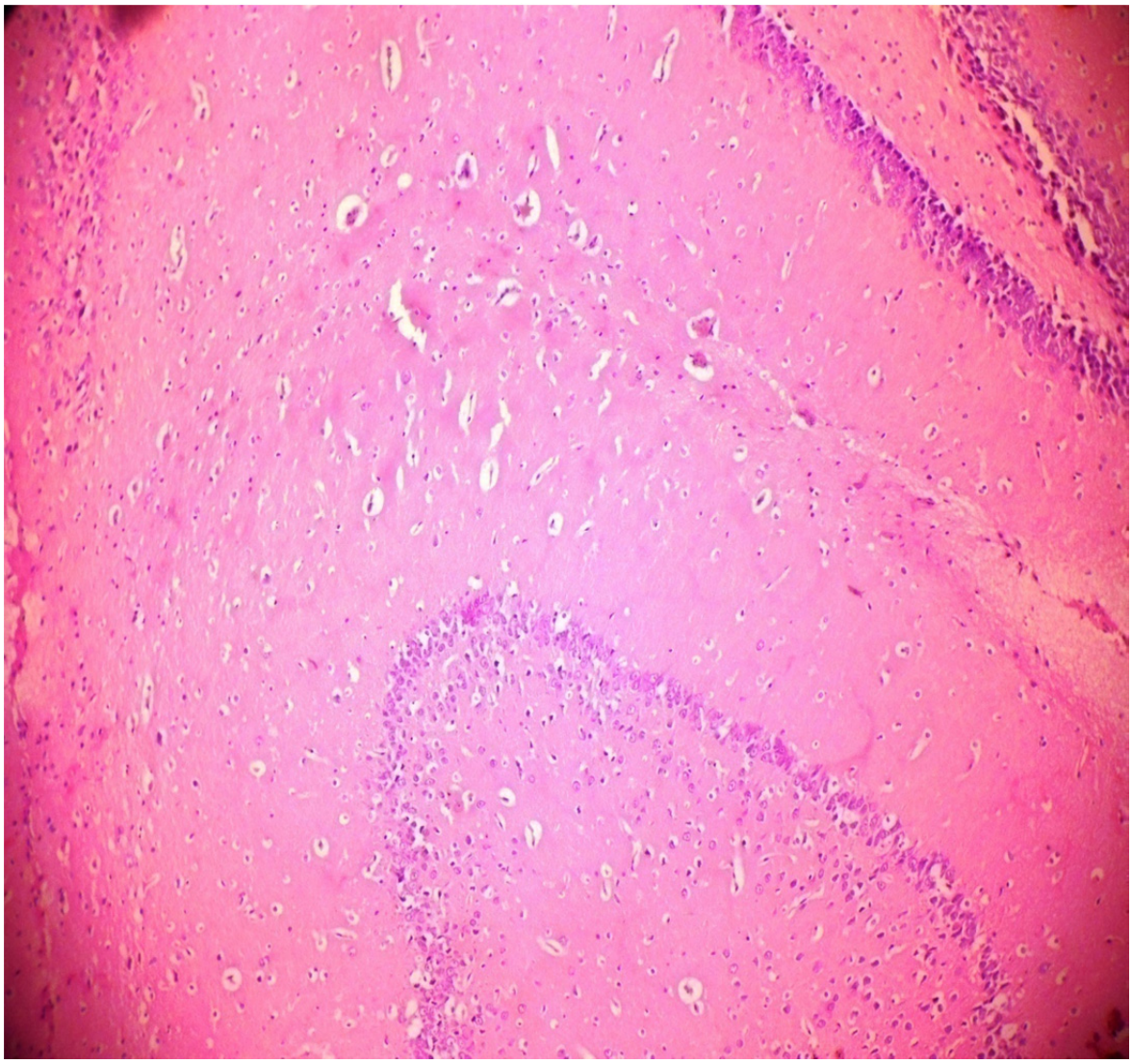


Fig No:19

G4: TREATMENT CONTROL

(20mg/kg MPTP+200mg/kg

HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD)

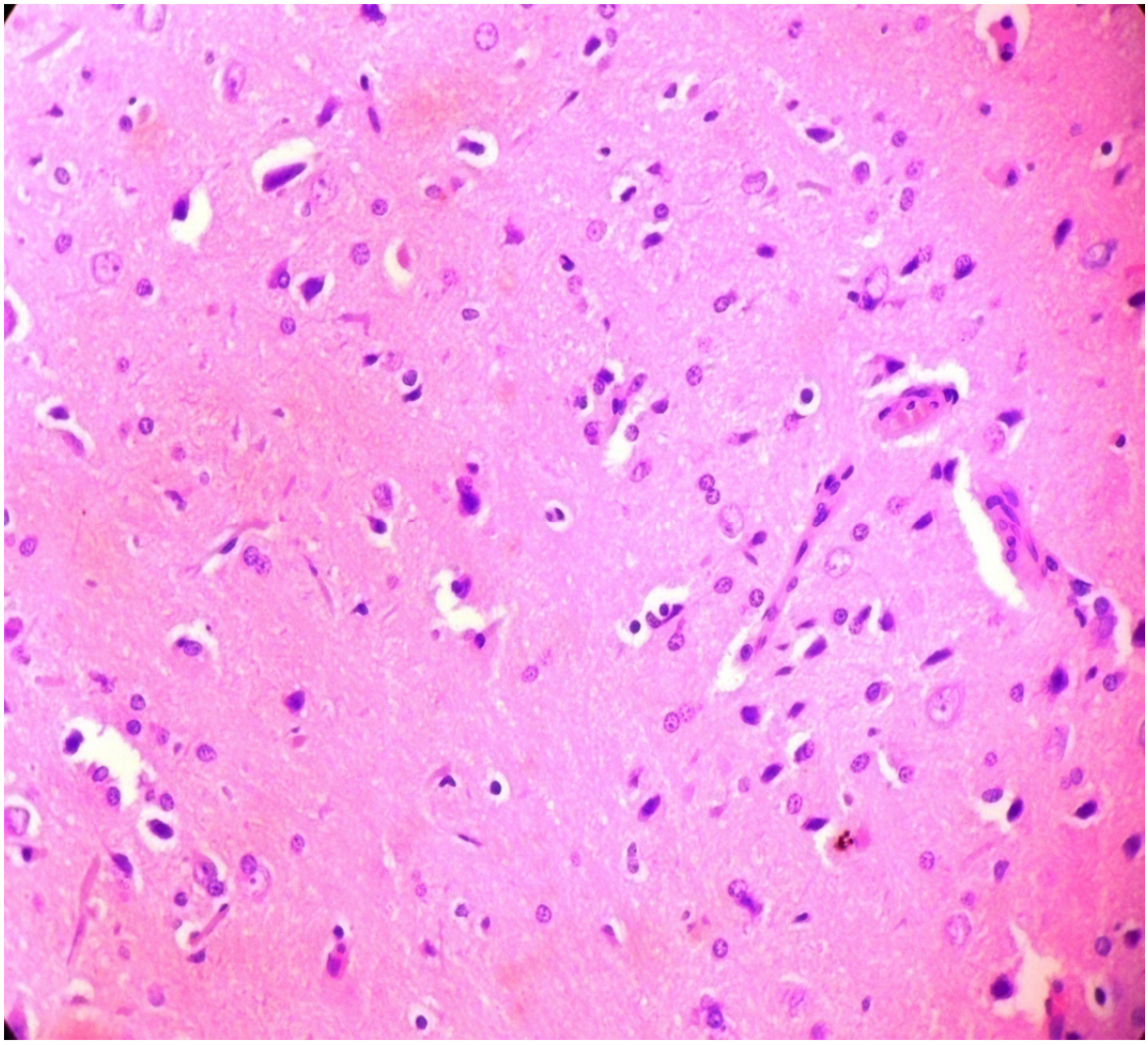


Fig no ;20

G5: TREATMENT CONTROL

(20mg/kg MPTP +400mg/kg

HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA (HAEBD))

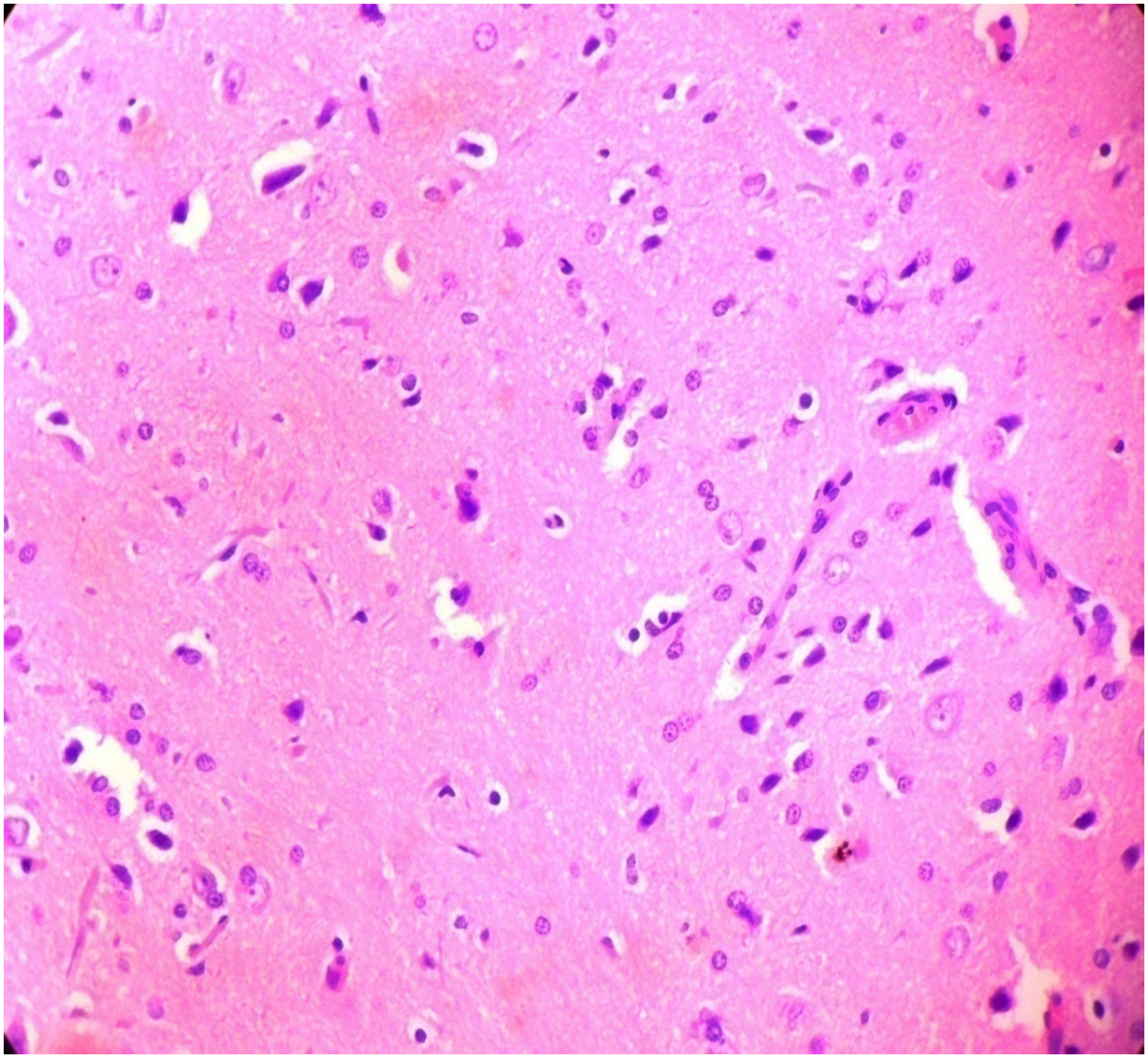


FIG NO:22

DISCUSSION

MPTP is a neurotoxin that produces a parkinsonian syndrome in both humans and experimental animals.[121] In the present study, the administration of MPTP significantly results in oxidative damage and mitochondrial dysfunction in brain. Furthermore, MPTP is rapidly converted to the hydrophilic metabolite 1-methyl-4-phenylpyridinium (MPP+) by MAO-B in astrocytes.[122] MPP+ is selectively accumulated by high affinity dopamine transporters (DAT) and taken up into the mitochondria of dopaminergic neurons, where it disrupts oxidative phosphorylation by inhibiting complex I (NADH-ubiquinone oxidoreductase) of the mitochondrial electron transport chain.[123] This leads to impairment of ATP production, elevated intracellular calcium levels, and free radical generation, thereby exhibiting dopaminergic neurotoxicity.[124] Therefore, MPTP treatment is known to cause a marked depletion of dopamine and nigrostriatal neuronal cell death in a wide variety of animal species, From these findings, we suggest that the dopamine depletion caused by the acute treatment with MPTP in rats is accompanied by sustained nigral degeneration.[125] Daily administration of *Boerhaavia diffusa* Linn extract at the dose of 200 mg/kg and 400 mg/kg p.o. reversed these behavioral and biochemical alteration induced by MPTP. The possible mechanism of neuroprotection conferred by Boerhaavia diffusa in behavioral and neurochemical parameters is the reduction of the oxidative stress caused by MPTP.

Glutathione a potent antioxidant, plays an important role in the dopamine and pathogenesis of Parkinson's disease presenting in the reduced form within the cells. It has been shown to react with free radicals and prevent generation of hydroxyl free radicals [1. Treatment with MPTP also leads to reduced activity of GPx and decreased levels of the essential pyridine nucleotide NAD^+, ATP, and GSH in primary human neurons after a 24-hour exposure. The maintenance of GPx activity appears crucial for the maintenance of cell viability during oxidative insult.[126-129]

MPTP treated rats shows highly significant increase in LPO levels in brain tissue homogenate. There was also a significant decrease in TA levels with MPTP treatment. The differences in the level of LPO products observed in various brain regions may be attributed to the differences in their iron content and diverse metabolism, which influence the generation of ROS. Certain brain regions like striatum and hippocampus are highly enriched with non-heme iron, which is catalytically involved in the production of ROS.[130]Exposure to MPTP might have lead to the peroxidation of membrane lipids eventually leading to the loss of membrane integrity and finally lead to cell death in these brain regions.

During oxidative stress in the neuronal cells there is an increase in intracellular calcium levels in the brain.[131]This increased intracellular calcium levels can induce the irreversible conversion of Xanthine dehydrogenase (XDH) to XO, which in turn catalyzes the oxidation of Xanthine to provide a source of oxygen. In addition, auto-oxidation of dopamine in brain could also serve as a source of superoxide anion.[132]These mechanism could be the main reasons for the increased levels of XO and reduction in activity of SOD leading to an overload of oxygen radicals and repression of antioxidant enzymes with MPTP exposure. Oxidation of L- 3,4-dihydroxyphenylalanine (L-DOPA) and dopamine (DA) to generate semiquinones/Quinones, oxygen radicals and other ROS may play a vital role in neuronal cell death in Parkinson's disease.

MDA is widely used to assess lipid peroxidation both *in vitro* and *in vivo*.[133] However, it is likely that MDA can form complexes with other biological components such as protein, lipids, and nucleic acids which can contribute to an underestimation of endogenous lipid peroxidation.[134] On the contrary to our lipid peroxidation data, we also show that MPTP can lead to distinct alterations in endogenous antioxidant defense mechanisms. MPTP treatment has been previously shown to significantly increase Mn-SOD and CuZn-SOD activities in the striatum of rats which is suggestive of acute oxidative stress insult.[135] SOD is upregulated in cells when $O_2^{\bullet-}$ is produced in excessive levels.[136]This observation suggests that SOD may play a role in the toxicity observed following acute treatment of MPTP, although ROS formation may not play a major role in MPTP-induced toxicity. We also observed a significant increase in CAT after a 24-hour treatment with MPTP. CAT is an enzyme that is involved in the detoxification of

ROS and the elimination of hydrogen peroxide (H_2O_2) in particular.[137]The increase in both intracellular SOD and CAT activities may therefore represent an adaptive response due to the leakage of free radicals during impaired mitochondrial respiration. Taken together, our data suggests that MPTP exposure can limit the endogenous antioxidant defense, subsequently increasing the vulnerability of neuronal cells to additional oxidative stress. An imbalance in the function of endogenous antioxidant defense mechanisms can lead to the accumulation of free radicals and ROS and increased susceptibility to oxidative stress, which contributes to the pathogenesis of PD.

Further, histological examination revealed that MPTP administration showed significant alterations in the neuronal architecture of striatum. Also, MPTP-induced lesions were present in large number, which are similar to the clinical anatomical abnormality seen in the Parkinson's disease patients.[138]HAEBD treatment prevented these neuro-architectural changes. Thus, it confirms Hydroalcoholic Extract of *Boerhaavia diffusa* offered protection to the dopaminergic neurons by preventing the histological changes induced by MPTP. Thus the *Boerhaavia diffusa* Linn extract at the dose of 200 & 400mg/kg p.o. showed a significant Neuroprotective activity

CONCLUSION

In summary, findings of the present study provide evidence that HAEBD at the dose of 400mg/kg p.o alleviates behavioral deficits. It also reduced the oxidative parameter and enhances the brain antioxidants, significantly prevents the brain from neurotoxic effects. Therefore, Hydroalcoholic extract of Boerhaavia diffusa Linn contains flavanoids and polyphenols was considered as powerful neuroprotective agent could offer a useful support to the Parkinsonism therapy.

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