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# EVALUATION OF NEUROPROTECTIVE ACTIVITY OF METHANOLIC LEAF EXTRACT OF *PHYLLANTHUS ACIDUS* IN CHLORPROMAZINE INDUCED PARKINSON ANIMAL MODEL

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## ABSTRACT

The present study is aimed evaluated the anti-Parkinson's activity of methanolic extract of Phyllanthus acidus (PAME) leaves in chlorpromazine(CPZ)-induced Parkinson animal model. The fruit extract was analysed to identify the phytochemical constituents present. Phytochemical screening revealed the presence of carbohydrates, steroids, saponins, triterpenes, phenols, flavonoids, and alkaloids. The anti-Parkinson's activity was studied in CPZ (3 mg/kg) induced Parkinson animal model. Rats were subjected to treatment with PAME and standard for a period of 21 days.The animals were given the PAME extract in a concentration of 200 mg/kg and 400 mg/kg. The effects of Phyllanthus acidus were studied using in vivo behavioral parameters like catalepsy and locomotor activity and its effects on neurochemical parameters like Dopamine, Catalase, brain SOD, and GSH in rats.Histopathology of brain was performed. PAME (200mg/kg and 400mg/kg) treated groups significantly reduced catalepsy and improved locomotor activity. They also showed an increase in the levels of Dopamine, brain SOD, GSH and decreased lipid peroxidation. Thus, the study proved that Phyllanthus acidus treatment significantly attenuated the motor defects and also protected the brain from oxidative stress. So, it may be concluded that the extract of Phyllanthus acidus leaves demonstrated a good antioxidant and neuroprotective effect in chlorpromazine induced Parkinson animal model.

**KEYWORDS:** Phyllanthus acidus, Methanolic extract, Catalepsy, Locomotor activity, Dopamine, Scopolamine, Anti-oxidant, Neuroprotective.

## INTRODUCTION

### Neurodegenerative diseases

Neurodegenerative diseases are the disorders of the brain with loss of neuronal activity. These are devastating and incurable which results in the loss of nerve cells or progressive degeneration in the human brain. This causes problems with mental functioning (dementia) or with movement (ataxias).

The actual cause of various neurodegenerative diseases still remains a mystery in health science. Some of the most studied environmental factors cause for neurodegenerative diseases are oxidative stress, inflammation, protein degradation, defects of mitochondria, due to higher activity of enzymes degradation of neurotransmitters in the synaptic cleft and abnormal protein accumulation in neuron.[1,2] One of the major problem of neurodegenerative diseases,[3] ageing.

Protein particles are delivered in the endoplasmic reticulum and furthermore in the cytosol. The polypeptide atoms which don't overlay appropriately are debased by ubiquitin proteosome framework and autophagy.[4,5] Cell apoptosis achieved by oxidative pressure which destructs the organic atoms and prompts cell death.[6-8] The current medicines for NDD's are restricted to giving suggestive help to early sickness. However, don't reduce harm to the neurons.[9]

### Parkinsons Disease

### History of Parkinson's Disease (PD)[10]

There are a few early writings that give proof that side effects of parkinsons disease were recorded in the Egyptian papyrus, the Ayurvedic composition the Bible. Old Indian writings from the 10,000 BC gave a few portrayals which looked like PD. Albeit a few creators had given part depictions of the illness, James Parkinson gave the main away from the record of PD. He composed

the paper "Exposition on the shaking palsy" in year 1817 where he portrayed six cases and gave clinical picture, progress disease with time, conditions like resting quakes, stride, strange stance and declining muscle quality. Jean-Martin Charcot included additional data of sickness and separated among unbending nature, shortcoming and bradykinesia in the year 1872 In the year 1911, Casimir Funk delivered the main enemy of PD drug, Levodopa. Fredric Lewy in 1912 clarified infinitesimal substances in influenced cerebrums, which was forthcoming named as "Lewy bodies". The fundamental cerebral part of our mind "substantianigra" is influenced in Parkinson's ailment clarified the noteworthiness of dopamine on PD. In the exact year, Spillantini, Trojanowski, indicated that alpha-synuclein is an essential part of Lewy bodies. The FDA permitted the use of medication Levodopa with Rasagiline in the year 2006 for patients with cutting edge PD. In 2017, as an extra treat, the medication Safinamide was approved by the FDA for Parkinsons patients on Levodopa or Carbidopa prescriptions encountering "off" scenes.

**Introduction**

Parkinson's disease is a neurodegenerative movement syndrome of the central nervous system which is progressive and chronic. Which occurs because of a decrease of dopamine-producing cells in the brain. This loss unfolds in substantianigra pars compacts (SNpc) of midbrain Which reduces the dopamine in the striatum. The other residual cells show intracytoplasmic "Lewy bodies."[12] These Lewy bodies contain Alpha-synuclein, may guide it to different cytotoxic mechanisms which may cause neurodegeneration.[13] As neuronal cells expire, four fundamental signs of PD appear, they include

- Rigidity
- Rest tremors
- Balance impairment or postural instability
- Bradykinesia or slowness of movement.

Dopamine (DA) is one of the chief neurotransmitters of the central nervous system(CNS) and a significant portion of DA to the brain is provided by mesodiencepgarlic dopaminergic neurons. The decrease of neurons in this part and midbrain Precedes movement impairment. The losing of DA is accepted to come near due to aggregation of Protein, Oxidative stress, mitochondrial dysfunction and decrease in neurotrophic signaling.[13,14]

Parkinson's disease happens all the more generally among the older populace with 1% above the age group of 60 years to 4% above the age group of 80, in spite of the fact that children and adults may also be affected. The etiopathogenesis of PD remains uncertain in spite of broad examination. There is developing confirmation that the death of neurons inside SNpcis attributable to apoptosis.[15]

Levodopa and Dopamine agonists endure the drugs used, alleviate the symptoms of Parkinsons Disease yet some drug treated patients may still progress the functional disabilities.[16]

**Epidemiology**

Parkinson's disease is a common neurological disease that marks at any time 1-2 per 1000 of the populace.[17] The frequency, the occurrence increases with increasing age and affect 1% of individuals beyond 65 years.[18]

Early-onset Parkinson’s disease (EOPD) is termed as the onset of characteristics of PD before the age of 40 years and records for 3-5% of all cases of PD. It is additionally named as 'juvenile' (< 21 years of age) and 'Young-onset' PD (YOPD) it’s between the age of 21-40 years.[19]

The number of patients experiencing Parkinsons disease existing in the ten most populated nations of the world and the five most populated nations in western Europe was approximately to be 4.1 and 4.6 million in 2005. This was recognized as 6.2 in 2015 and is likely to increase to 8.2 million by the year 2030. Likewise in Asia's six most populated nations, this amount will ascend from 2.57 million in 2005 to 6.17 million in 2030.[20]

In India, there are only a few large scales epidemiological statistics on PD. A study accomplished for neurological disorders in Kolkata indicated a occurrence of 45.82 per 100,000.[21] A rough occurrence of 33 for each 100,000 and age-adjusted- 76 in Bangalore and of 14.1 per 100,000 and age-adjusted prevalence - 134 per 100,000 was accounted in rural Kashmir.[22,23] Surveillance carried out in old age homes indicated that 109 out of 612 residents, i.e., 17.6% experienced Parkinson.[24] The Paris community of India living in Mumbai demonstrated a crude occurrence ratio of PD of 328.3 per 100000 Populace.[25]

**Etiology**

Parkinsons Disease is multifactorial in nature with age being a huge threat factor. The Definite aetiology of PD remains unclear in spite of efforts. The aetiology of PD may include different factors either genetic or environmental.[26]

**Genetic factors**

Mutations in different gene augment the probability of PD progress. Gross. Many genetic factors have been recognized as a risk to increase PD. Monogenetic form of PD usually caused by a alteration in a gene which may be recessively inherited or dominantly inherited. This caused sporadic PD about 3-5 % and familial PD about 30%.[27]

**Sporadic PD:** Interaction of genetic variant with different genes and environmental factors which causes PD.[28]

**Familial PD:** Mendelian inheritance, causes penetration mutations which leads to the development of the disease.

Transformations in these genes are either autosomal recessive or autosomal dominant. So far 23 genes have been related to Parkinson out of which PARK3, PARK10, PARK12, PARK16 & PARK22 are deliberated as threat factors and the contribution of PARK5, PARK11, PARK13, PARK18, PARK21 & PARK 23 have not been recognized.[29]

**Environmental factors**

Heavy metals, Herbicides, Pesticides and water-borne risk factors have been related to PD. Pesticides like parquet and rotenone have also been cognated. cognates. People living in rural areas where wells are utilized for water source are at high risk of developing PD.[30-32]

**Ageing**

The probability of ageing in the pathogenesis of Parkinson is recommended by its occurrence in late middle age and by marked increments in its prevalence at old age.[33] The possible Contribution of age to the Parkinson is further supported by earlier studies which showing a loss with the age of striatal DA and DA of cells in the SN[34]. However, while the Progressive loss striatal dopaminergic markers and SNpc neurons.[35] with age has been confirmed the pattern and timing of these losses contrast from what occurs in PD, indicating that ageing is not likely to play a direct role in the degenerative process.

**Caffeine**

It's been informed that tea drinkers and coffee drinkers have minimum probabilities of developing PD. Caffeine being an adenosine A2A receptor antagonist in PD has a neuroprotective effect in PD mouse model.[36-38]

**Neuropathology**

PD is usually an idiopathic condition but there are cases of PD occurring in people with a Mendelian inheritance or a family history. In idiopathic PD, the brain only shows ventricular dilation and mild atrophy of frontal cortex macroscopically. In PD transverse section of the brain shows definite alterations. There's deficit of dark pigmented area within locus-coeruuleus(LC) & SN pars compact(SNpc) ascribable to loss of neurons - noradrenergic within LC &death of neuromelanin containing DAergic( A9 neurons) in SNpc.[39,41]

**Lewy Body**

This is one of the pathological sign of PD. Which is termed as "Lewy body"

Lewy bodies(LBs) are the unusual cytoplasmic stores in the neuronal cell bodies that are immunoreactive for protein α- synuclein assumed as a pathological hallmark in PD.[42] These occurs with Lewy neuritis which is dystrophic neurites.[43,44]

LBs comprises of a granular and fibrillar core encompassed by a halo. The size of its ranges from 5 to 30μm in diameter and a single neuron contains >1 LB.[45]

Alpha-Synuclein isLBs fundamental component. α-synuclein is a filamentous protein found all through the brain, It forms an amyloid-like structure and becomes usually aglomerated and phosphorylated, Other types of synucleinopathies.[46-48] LBs additionally contains different proteins like ubiquitin, heat shock proteins, parkin, parking, tubulin, nitrated or oxidized proteins along with lysosomal and proteasomal elements.[49]

**Pathogenesis**

**α – Synuclein Misfolding**

The α - synuclein upon contact with negatively charged lipids it folds over its N - terminal to form structure i.e., α - helical structure.[50,51] α - synuclein in PD acquires an amyloid-like structure which is beta-sheet rich and prone to aggregate. This misfolded alpha- synucleins are found inside LBs as lengthy filaments (5-10 nm). Serine 129 phosphorylation, ubiquitination and C-terminal truncation have been recommended to cause the conformational changes that lead to alpha-synuclein aggregation.[52,53] The oligomeric form of alpha-synuclein are neurotoxic, accelerates the abnormal protein aggregation.[54,55,56]

**Dysfunctional protein clearance systems**

The dualistic protein clearance systems which are responsible for eliminating dysfunctional proteins are specifically-

1. Ubiquitin- proteasome system (UPS): The UPS break downs abnormal proteins by labelling them with ubiquitin. It at that point transports for degradation to the proteasome.
2. Autophagy-lysosome pathway

- Microautophagy: The lysosome envelopes and destroys cytoplasmic substance in microautophagy
- Macroautophagy: The components are surrounded by autophagosome that joins with the lysosome and then breaks down its substances.
- Chaperone Mediated Autophagy (CMA): In this, chaperones target particular proteins and is transported for degradation to the lysosome.[57]

Any destruction to either the Ubiquitin-proteasome system or the autophagy-lysosome pathway may be involved in pathogenesis by accumulation defective proteins particularly the misfolded alpha- synuclein.[58,59]

**Signs and Symptoms**

**Motor manifestations**

The 4 main manifestations of PD are tremors, bradykinesia (slowness), rigidity or stiffness & postural instability.[60]

- Tremors: one of the common feature of Parkinsons Disease. Slow tremor of hand which occurs at a frequency between 4&6 Hz. They take place in one hand usually but as the disease increases, both of the hands are affected.'Pill rolling' is a feature where the thumb and the forefingers tend to trace each other and perform the circular movement.

- Bradykinesia: Slowness in movement. Every aspect of movements i.e., planning - initiation - execution.
- Rigidity: Contractions of muscle, stiffness and resistance to the movement of limbs.
- Postural instability: It arises in later stages of PD. Impaired balance and falls are frequent.
- Other motor manifestations accompanying PD include,
- Mask like facial expression, Monotonous voice and slurred speech
- Gait: Decreased arm swing, rapid shuffling steps
- Emotional changes, difficulty with swallowing
- Depression
- Urinary problems
- Sleeping problems
- Orthostatic hypotension
- Dystonia
- Hallucinations and additional psychotic symptoms
- Muscle cramps
- Fatigue
- Neuropsychiatric manifestations:
- Delusions of Hallucinations
- Impulse control disorders
- Depression, apathy and anxiety
- Punding: Repetitive erratic behaviours occurring triggered by medicines taken for the treatment of PD.
- Dementia: Patient practices difficulties with Memory, thought, language and social judgement. Medicines taken for the treatment of motor signs may cause confusion and hallucinations.
- Cognitive disturbances: It includes complications with flexibility in cognition, planning performing actions, difficulty in the estimation and perception of time and visuospatial difficulties.

**Other manifestations**

In addition to these neurological signs and symptoms, PD patients frequently have disturbing sensory symptoms and pain in affected limbs.

**Many PD patients also have signs of**

- Autonomic failure
- Orthostatic hypertension
- Constipation
- Urinary problems
- Impotence in men

**Diagnosis of PD**

The diagnosis is most difficult because there's no particular cause of emerging PD. Early identification may help to begin the treatment and a better standard of living since the early signs are missed. The patient suffering > 2 symptoms like tremors, slow movements, stiffness, freezing of shoulders, micrographic, anosmia, voice change and disturbances in sleep should consult a neurologist.[61,62] Even though advances in PD management, there is no particular biological or laboratory test, the radiological technique for diagnosing PD and hinges the four primary signs to levodopa usage.[63]

Diagnostic investigation: The variations in DA in the brain can be performed using brain scans.

Neuroimaging techniques

**1. Magnetic Resonance Imaging (MRI) Scan**

MRI scans provide a advanced view of the deep anatomy of the brain. It differentiates the conditions that mimic Parkinsons Disease like Progressive supra nuclear palsy (PSP) and multiple system atrophy (MSA). This uses magnetic currents to produce images of the brain.

**2. Computerized Tomography (CT) Scan**

CT scans give a imagining brain and rules out blood infections or tumour's that mimic PD. A radioactive dye in this practice, is injected into that coheres neurons that release DA and signals are noted.

**3. Dopamine transporter (DAT) Scan**

Imaging of DA apparatus in the brain is achieved by Data Scan. In this technique, a radioactive dye when injected into the body assigns to the DA system in the brain and records the indications of DA releasing neurons.

**Treatment**

There's no complete cure for PD. The present treatments are focused on providing symptomatic relief.[64]

The drugs used for the treatment of PD are given in the following table

**Table 1: Classification of Drugs being used to treat PD.**

| S. No. | Drug Classification | Adverse effects |
|---|---|---|
| 01 | Drugs enhancing the levels of DA in the brain Ex. Levodopa, Carbidopa | low BP, Nausea, Vomiting, drowsiness, psychosis, restlessness and hallucinations. |
| 02 | Drugs inhibits the action of DA<br>a) MAO-B inhibitors Ex. RasagilineSelegiline.<br>b) COMT inhibitors Ex.Entacapone, Tolcapone | Nausea, insomnia, orthostatic hypotension Diarrhoea, dizziness, Nausea, low BP, Disturbances in sleep, abdominal pain, liver disorders, hallucinations. |
| 03 | Agonists- Dopamine<br>Ex. Apomorphine, Pramipexole, Ropinirole | sudden sleep onset, Nausea, confusion, nightmares, hallucinations. |
| 04 | Drugs declining the cholinergic (Ach) activity<br>Ex.Benztropine,Trihexyphenidyl, Ethopropazine | Blurred optical vision, dry mouth, urine retaining, memory loss and confusion. |
| 05 | Drugs with an unidentified MOA Ex. Amantadine | insomnia, agitation and hallucinations. |

**Surgery**

Parkinsons diseased Patients to whom the drug treatment is not satisfactorySurgerical therapy has opted.

1. **Pallidotomy:** Pallidotomy surgical process encompasses the destroying of globuspallidus a segment of the brain. It affects with the connections between striatum or thalamus and globuspallidus and improves rigidity, tremors and bradykinesia.
2. **Thalamotomy:** In this procedure, segment thalamus is damaged thus improving tremors. Due to the permanent destruction of tissues, Thalamotomy is replaced with deep brain stimulation.
3. **Deep brain stimulation:** In the subthalamic nucleus or globuspallidus of the brain, an electrode is embedded via surgery which collects signals from a pulse generator in the chest as it blocks the signals causing motor symptoms of Parkinsons disease without any pain.[65]

**Plant Profile**

**Botanical synonyms:** Phyllanthusdistichus, CiccaacidaMerr, Ciccadisticha Linn, Averrhoaacida Linn.

**Figure 1: *Phyllanthus acidus* plant.**

**Taxonomical classification[66]**

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Euphorbiales
Family: Phyllanthaceace
Genus: Phyllanthus
Species:acidus
Parts used: leaf

**Vernacular Names**

Hindi: Chalmeri, Chotaaonla, Harfauri, Harparauri.
Urdu: Harfarauri.
Bengali: Hariful.
Jamaica: Jimbilin.

**General Description**

- Phyllanthusacidus is widely distributed in India and other Asian countries.
- The height of the plant is about 4-6m high with obliquely ovate acute and distichous thin leaves.
- This plant is an intermediary between shrubs and trees.
- It has edible small yellow berries fruits which are tart and sour belonging to phyllanthaceae family.
- These are a rich source of secondary metabolites and various saponins and alkaloids.

**Origin**

- This tropical & subtropical species is found all through Asia and furthermore in the Caribbean district, Central and South America.[67]
- Its origin is dubious, the species may have begun in Madagascar. It was found in different pieces of South Asia ahead of schedule; as per Eduardo Quisumbing, it was brought to the Philippines in ancient times.[68] It spread over the Indian Ocean to Réunion and Mauritius and crossed the Pacific to Hawaii. It extended to the Caribbean in 1793, when William Bligh conveyed the plant from Timor to Jamaica.[69]
- Chemical constituents
- Main chemical constituents of the leaf of phyllanthusacidus include kaempferol, gallic acid, hypogallic acid, quercetin and adenosine.
- It contains derivatives of epicatechin,coumaric, quercetin and cinnamic acid.
- These are also a rich source of metabolites like terpenes, alkaloids, saponins, flavonoids, anthraquinones.

**Uses**

- Leaf is analgesic, antipyretic, antirheumatic and cures Jaundice, smallpox, itching and gum infection.
- Traditionally it is used as liver toxic and blood purifier

- The medicinal activities of Phyllanthus species are antipyretic
- Leaves of *P.acidus* also used as a hepatoprotective drug in which it inhibits α-glucosidase.
- Leaves also exhibits antimicrobial and anti cytotoxic activity.

## LITERATURE REVIEW

**Jain et al., 2011,**[70]evaluated the hepatoprotective activity of the ethanol extracts of the fruits of Phyllanthusacidusin CCl4-induced liver injured mice and rat models. The ethanolic extract decreased the total serum levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TB), alkaline phosphatase (ALP), and lipid peroxidation (LPO); and increased in the levels of total protein (TP), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) in comparison to the control groups. It was found effective at a very large dose of the extract i.e., 400 mg/kg, PO was found to have a comparable protective effect with the reference drug silymarin (100 mg/kg, PO).

**Jain and Singhai, 2011; Jain et al., 2010,** reported the hepatoprotective property of P. acidus fruit extract at three doses i.e., 125, 250, and 500 mg/kg, PO, and P. acidus leaf extract at two doses i.e., 200 and 400 mg/kg, PO Thioacetamide-induced and Acetaminophen-induced liver injured rat models respectively. The outcomes of this study indicated the significant prevention of the harmful effects of thioacetamide and acetaminophen specifically at a high dose of 400 mg/kg, PO, followed by a recovery to normal by Phyllanthusacidus extracts.

**AbdGhafar et al. (2018)**[71] evaluatedtheglucosidase inhibitory activity of ethanolic extracts of leaves and fruits of Phyllanthusacidus. The results indicate remarkable α-glucosidase inhibitory activity, moderate nitric oxide scavenging activity, and nitric oxide inhibitory activity atIC50 values of 2, 160, and 180 µg/mL, respectively, and with the highest total phenolic content of 33 mg GAE/g leaf extract.

**AbdGhafar et al., 2018;** Sulaiman and Ooi, 2014, performed ultra-high-performance liquid chromatography (UHPLC) analysis of Phyllanthusacidusfruit extract and quantify the phenolic compounds in the fruit extract such as gallic acid, quercetin,dihydroquercetin, kaempferol, and myricetin.

**Brainina et al., 2019,** evaluated the antioxidant potentials of the extracts of the leaves, bark, and fruits of thePhyllanthusacidus is based on the in vitro free radical scavenging capabilities in contrast to DPPH, hydroxyl radicals, superoxide radicals, lipid peroxidation, metal chelating assay, etc. The presence of antioxidant constituents in the extracts, such as phenolic and flavonoid compounds, served as scavengers for reactive oxygen species.

**Jagessar et al., 2008,**[72] evaluated the antimicrobial activity of the aqueous extract of Phyllanthusacidus against a large spectrum of bacteria and fungi. The aqueous extract of the leaves showed potential antimicrobial activities towards E. coli, Candida albicans, and Staphylococcus aureus by using the disc diffusion method with the zone of inhibition of 22mm, 20mm, and 21 mm, respectively, compared to the standard reference (Ampicillin for E. coli and S. aureus; nystatin for C. albicans).

**Jagessar and Hope, 2016,** reported moderate antimicrobial activity of leaf extract against S. aureus, E. coli, C. albicans, and Pseudomonas aeruginosa with the average zone of inhibition of 23mm, 15mm, 11mm, and 10 mm, respectively.

Padmapriya and Poonguzhali, 2015, evaluated the antimicrobial activity of acetone fruit extract of Phyllanthusaciduswiththe average zone of inhibition ranged from 13mm to 20 mm in contrast to the growth of E. coli, Bacillus cereus, P. aeruginosa, andS. aureus. The highest inhibitory action of the extract was establish against the growth of E. coli and P. aeruginosa with the average zone of inhibition of 19mm and 20 mm, respectively.

**Vongvanich et al., 2000,**[73] evaluated the cytotoxic activity on isolated compounds of Phyllanthusacidusextractby several groups. The reported norbisabolane glycosides, phyllanthusol A and B, demonstrated cytotoxic activity against BC cell line with EC50 at 4 µg/mL for both; and KB cell line with EC50 at 15 and 9 µg/mL, respectively, in comparison to the standard drug ellipticine with EC50 at 0.3 µg/mL for both cell lines.

**Moniruzzaman et al. (2015),** reported that the methanolic fruit extract of Phyllanthusacidusshowed inhibition of acetylcholinesterase in the brain cells of rats and human blood butyrylcholinesterase in vitro in a dose-dependent fashion with an IC50 value of 1010 µg/mL and 450 µg/mL, respectively. The screening was executed as per Ellman et al. (1961) and indicated that this extract has a significant anti-acetylcholinesterase and anti-butyrylcholinesterase activity, against the standard drug, donepezil with IC50 values at 32µg/mL and 17 µg/mL, respectively.

## AIM AND OBJECTIVES

### Aim

To evaluate the neuroprotective activity of methanolic leaf extract of phyllanthusacidus in CPZ induced Parkinson animal model.

### Objectives

- To evaluate the neuroprotective activity of methanolic leaf extract of phyllanthusacidus in CPZ induced Parkinson animal model.

- To measure the behavioural changes such as rigidity, tremors, locomotor activity, postural changes and altered movements by performing
- Catalepsy test
- Locomotor activity test

To measure parameters of brain homogenates
- Dopamine
- Lipid - peroxidation
- Reduced Glutathione
- Superoxide - dismutase
- Histopathological studies

**PLAN OF WORK**

1. Identification and collection and authentication phyllanthusacidus (leaf)
2. phyllanthusacidus leaves methanolic extract(PAME)
3. Phytochemical screening of the extract
4. Collection and procurement of Wistar albino rats
5. Grouping the experiment animals into 5 groups comprising 6 animals in each group.

1) Control Group: 0.5% w/v CMC by oral route.
2) Disease Control: Chlorpromazine 3mg/kg body wt. i.p. in 0.5% w/v CMC.
3) Standard group: Tab. Syndopa 110 (Levodopa + Carbidopa) 10mg/kg body wt. oral in 0.5% w/v CMC + Chlorpromazine 3mg/kg body wt. i.p. in 0.5% w/v gum acacia.
4) Test Dose 1: Methanolic extract 200mg/kg body wt. oral + Chlorpromazine 3mg/kg body wt. i.p. in 0.5 % w/v CMC.
5) Test Dose 2 :Methanolic extract 400mg/kg body wt. oral + Chlorpromazine 3mg/kg body wt. i.p. in 0.5% w/v CMC.

6. Behavioural assessment of animals
7. Biochemical evaluation from Brain homogenates
8. Histopathological studies
9. Result & Discussion
10. Conclusion

**MATERIALS AND METHODS**

**Drugs and chemicals**

- Methanol,S-D fine chem. Pvt. Ltd.
- Benedict's Reagent, Universal laboratory.
- Fehling's Reagent A& B,S-D fine chem. Limited, Mumbai.
- Salwinoff's Reagent,Nice chem. Pvt. Ltd.
- Barfoed's Reagent,Nice chem. Pvt. Ltd.
- Molisch Reagent,Nice chem. Pvt. Ltd.
- Tollin's Reagent,Nice chem. Pvt. Ltd.
- Potassium Hydroxide,S-D fine chem. Labs.
- Hydrochloric Acid,S-D fine chem. Pvt. Ltd.
- Sodium Hydroxide,S-D fine chem. Labs.
- Sulphuric Acid,S-D fine chem. Labs.
- Ammonium Sulphate, Merck Labs.
- Hydrochloric Acid,S-D fine chem. Labs.
- Lead Acetate, Accorel Labs.
- Sodium Carbonate,S-D fine chem. Labs.
- Mayer's Reagent, B.B Labs.
- Wagner's Reagent,Nice chem. Pvt. Ltd.
- Ammonia, S-D fine chem. Labs.
- Dragon Droff's Reagent,Nice chem. Pvt. Ltd.
- Calcium Chloride, Merck Labs.
- Gelatin, Nice chem. Pvt. Ltd.
- Potassium Permanganate, S-D fine chem. Labs.
- Sodium Bicarbonate, S-D fine chem. Labs.
- Sodium Chloride, S-D fine chem. Labs.
- Chloroform, S-D fine chem. Labs.
- Dipotassium hydrogen phosphate, S-D fine chem. Pvt. Ltd.
- Dipotassium hydrogen orthophosphate, S-D fine chem. Pvt. Ltd.
- Sodium Hydroxide, S-D fine chem. Pvt. Ltd.
- n - Butanol, NR chem
- Hydrochloric acid, S-D fine chem. Pvt. Ltd.
- Sodium acetate, S-D fine chem. Labs.
- Glacial acetic acid, S-D fine chem. Pvt. Ltd.
- Potassium iodide, S-D fine chem. Labs
- Sodium Hydroxide, S-D fine chem. Labs.
- Ethanol, S-D fine chem. Pvt. Ltd.
- Iodine, S-D fine chem. Pvt. Ltd.
- Sodium thiosulphate, S-D fine chem. Labs.
- Sodium Hydroxide, S-D fine chem. Pvt. Ltd.
- Acetylthiocholine iodide, National chemicals.
- DTNB Reagent, National chemicals.
- EDTA, National Chemicals.
- Hydrogen peroxide, S-D fine chem. Pvt. Ltd.
- Sodium carbonate, S-D fine chem. Labs.
- Sucrose,S-D fine chem Pvt. Ltd.
- Adrenaline, pharma Cure Labs.
- Acetic Acid,S-D fine chem. Pvt. Ltd.
- Thiobarbituric acid, National Chemicals.
- Pyridine,S-D fine chem. Pvt. Ltd.
- Sodium lauryl sulphate,S-D fine chem. Pvt. Ltd.
- Trichloroacetic Acid,S-D fine chem, Pvt. Ltd.

**Methodology**

**Sample – Collection and Authentication**

The leaves of phyllanthusacidus were collected and authenticated by Dr Shaik Mohammed Aliuddin, (Secretary, Hyderabad Unani Research Foundation, Hyderabad, Telangana) in the month of December 2019.

**Processing of sample**

The procured samples were grounded into gunpowder and used for extraction.

**Figure 2: crude powder form of sample.**

**Preparation of extracts**

Soxhlet method was employed for the preparation of the methanolic extracts.

**Method:** The crude preparation samples was finely ground, were blended and put in the Soxhlet apparatus chamber. The round base flask was loaded up with the extracting solvent i.e. methanol and warmed while its fumes get consolidated in a condenser. The solvent which is reduced droplets onto the cover with the crude powdered drug and extracts on coming in interaction with it. At the point when the liquid in the chamber gets risen to the top, the fluid substance is then sent to the solvent comprising flask. This constant phase is performed till the siphon tube dissolvable drop leaves not any more evaporative residue. To abstain from knocking during warming, the round bottom flask is loaded up with boiling chips.[74]

**Figure 3: Preparation of extract using soxhlet apparatus.**

**Preparation of PAME**

500gms of grounded plant drug was extricated with 1000ml of Methanol utilizing soxhlet apparatus in various batches. The obtained extraction was then subjected to water bath at 80° to attain *Phyllanthusacidus* Methanolic extract (PAME).

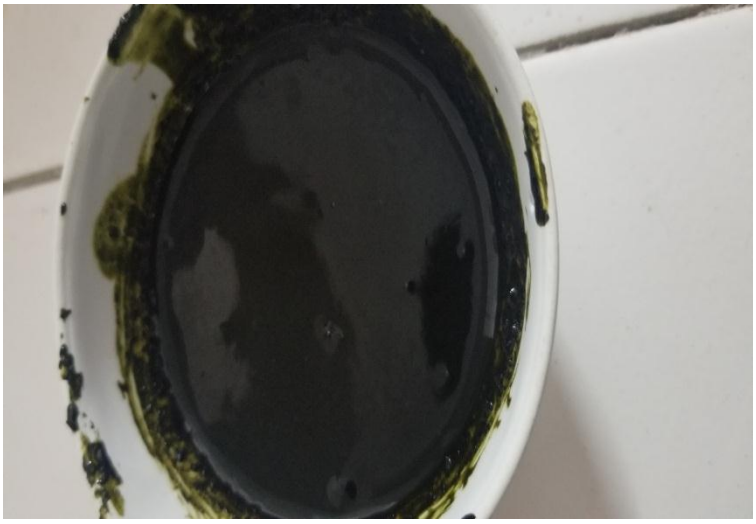

**Figure 4: Extracted sample of *Phyllanthusacidus.***

**Phytochemical Investigation of Pame**

**1. Tests for carbohydrates**

**a. Benedict's test**

Equivalent volumes of PAME and benedict's reagent were blended& was warmed in a water bath. Reducing sugars were present, test solution looks green or yellow or red.

**b. Fehling's test**

Fehling's A and Fehling's B solutions of about 1ml each were blended& boiled for one min. An equal volume of PAME was added & warmed for 5-10 min in boiling water bath. Observance of yellow or red brick colour ppt.

**c. Molisch's test**

To 2ml PAME, few drops of α-naphthol alcoholic were added then shaken and conc. $H_2SO_4$ was added from sides, the violet ring is formed.

**d. Barfoeds test**

Equal volumes of both PAME and barfoed's reagent were taken , warmed for 1-2 mins in a water bath and cooled. A red colour ppt is observed.

**2. Test for flavonoids**

**a. Sulphuric acid test**

Sulphuric acid was added to PAME. The presence of a deep yellow solution shows the presence of flavonols& flavones, red or bluish-red solutions indicate chalcones&aurones and Flavones show orange-red colour.

**b. Shinoda test**

PAME was additional with 5 ml 95% ethanol, 0.5 g Mg turnings and a few drops conc. HCl. The presence of Pink, orange, red, purple indicates derivatives such as xanthenes, flavonols.

**c. Lead acetate test**

To PAME lead acetate solution was added . Yellow ppt specifies the presence of flavonoids.

**3. Saponins Test**

**Foam test**

To the PAME, water was added and shaken. The presence of foam indicates the presence of saponins.

**4. Phenols and tannins Test**

**a. 5% $FeCl_3$ solution test**

Few droplets of ferric chloride were added to 2-3ml of PAME. The deep blue-black colour appears, indicates the presence of phenols and tannins.

**b. Lead acetate test**

Few drops of lead acetate soln. were added to 2-3ml of PAME. White ppt. is formed which indicates the presence of phenols and tannins.

**c. Bromine water test**

Few drops of Bromine water was added to 2-3ml of PAME. Discolouration of Br water occurs, denotes tannins and phenols.

**d. Acetic acid test**

Few droplets of acetic acid is taken with 2-3ml of PAME, Red colored solution specifies the presence of tannins and phenols.

**e. Dilute $HNO_3$ test**

Few droplets of dil. $HNO_3$ was added to 2-3ml PAME. Reddish to yellow coloured directs the presence of tannins and phenols.

**5. Tests for steroids**

Salkowski reaction: 2ml each of conc. Sulphuric acid and chloroform were added and shaked well to 2ml PAME. $CHCl_3$ confers red and $H_2SO_4$ layer gives greenish-yellow fluorescence.

**6. Test for alkaloids:** Separate evaporation of extracts was done and dil. HCl was added to the residue, it is shaken, filtered and performed the following with the filtrate.

**a. Dragendorff's test**
Few drops Dragendorff's reagent is added to 2-3 ml of filtrate. The appearance of orange-brown ppt. which shows the presence of alkaloids.

**b. Mayer's test**
Few drops of Mayer's reagent is added to 2-3 ml solution which gives ppt.

**c. Tannic acid test**
Tannic acid when treated with PAME extract gives buff coloured ppt.

**7. Tests for Proteins**
**a. Xanthoprotein test:** 3ml of PAME was added with conc. H2SO4. White ppt is formed which on heating turns yellow. This on the addition of NH4OH turns to orange colour.
**b. Biuret test:** To the 3ml PAME, 4% NaOH& few droplets of 1% CuSO4 were added. Violet or pink colour is observed.

**8. Tests for amino acids**
Ninhydrin test: The PAME extract 3ml was heated and added with few drops 5% ninhydrin soln. for 10 min. the purple or bluish colour is observed.

**Toxicological Report**
Although the roots of Phyllanthusacidus are considered as poisonous in Malaya, the data regarding the toxicity of Phyllanthusacidus is restrained to this point. The historical prescriptions and clinical reviews about the toxicity of the Phyllanthusacidus were also very rare. However, various researches on the fruit and leaf extracts of the Phyllanthusacidus had been evidenced to be safe and no harmful impact is seen in vitro and in vivo.

A report by Bagavan et al., 2011, confirmed no cytotoxic impact of the leaf extract towards Vero normal cell lines through MTT assay.[75]

Another report by Bhowmik et al. (2015) showed that no toxic effects or mortality was determined in the albino rats and mice after oral administration of the extract of leaves at a dose of 2000mg/kg for a period of 24 h.[76]

Likewise, Chaimum-aom et al., 2017; Jain and Singhai, 2011; Jain et al., 2011, reported the acute oral toxicity of ethanolic extract of fruit; and aqueous and ethanolic extracts of leaves at a dose of 2000mg/kg, PO, to be non-toxic and no mortality was seen in both albino mice and rats.[77]

Based on the above report, the PAME at an oral dose of 200mg/kg and 400mg/kg was chosen for pharmacological screening.

**Pharmacological Screening Methods**
**Experimental animals**
Adult Wistar Rats were taken for this study and procured from Sainath Animal Agency, Musheerabad, Hyderabad, India. Rats weighing 100-150gm were kept under standard well-controlled environment before and throughout the duration of experiment. The temperature was maintained at 22°C (±3°C) and relative humidity,50-60%. Pellet diet was feed and water ad libitum, The experimental procedure was approved by the Institutional Animal Ethical Committee and was accepted under the compliance of IAEC guidelines. (IAEC/SUCP/2019/07)

**Experimental design**
30 experimental rats of either sex were divided into 5 groups and treated as follows:

1. **Normal Control Group :** This group was given 0.5% w/v CMC vehicle and normal feed diet orally-21 days.
2. **Disease Control Group:** These are treated with CPZ (3mg/kg b.w.) i.p. dissolved in 0.5% w/v CMC -21 days
3. **Standard Group:** Tab. Syndopa 110 (10mg/kg b.w) in 0.5% w/v CMCi.p. + CPZ (3mg/kg b.w.) i.p. dissolved in 0.5% w/v CMC - 21 days
4. **Test dose (PAME) I group:** : Treated with test dose- 1 of PAME (200mg/kg b.w., p.o.) + CPZ (3mg/kg b.w.) i.p. dissolved in 0.5% w/v CMC for a period of 21 days.
5. **Test dose (PAME) II group:** This group was treated with test dose- 2 of PAME (400mg/kg b.w., p.o.) + CPZ (3mg/kg b.w.) i.p. dissolved in 0.5% w/v CMC- 21 days.

30 min prior to dosing of the syndopa and PAME, CPZ (3mg/kg b.w.) i.p. suspended in 0.5% w/v CMC was given to the standard and test groups.

Chlorpromazine (CPZ) was administered to induce Parkinsosns disease.

Parkinsons disease was initiated by, intraperitoneal administration of Chlorpromazine 3mg/kg body wt. suspended in 0.5% w/v CMC suspension induced PD.

**Behavioural Assessments For PD**
**1.Catalepsy (Block method)**
Principle:-The outcome of standard drugs and PAME on cataleptic behaviour of Chlorpromazine rats was performed on wooden block method steps are involved in this method are:
**First Step:** The rats were positioned on the table and gently touched on back.If the rat was not moved, a cataleptic score of 0.5 was provided.

**Second step:** Then the rat's front paws were positioned on a block of 3cm height. Then the score of 0.5 for each paw was noted and added it to the 1st step's score if rats doesnot move failed to correct in their posture in 15 seconds

**3step:** Alternatively, the rat's front paws were positioned on the wooden block of 9cm height. Then a cataleptic score of 1 for each paw was given and added it to the 1st and 2nd step's score if rats doesnot correct their posture in 15 seconds.

The cut-off score of rats was 3.5 and that indicated complete catalepsy.[78]

**Figure 5: measurement of catalepsy.**

**2. Locomotor activity (Actophotometer)**

Principle: Actophotometer comprises an infrared shaft. This shaft is digitally displayed after being recorded. It works with stand associated with photoelectric cells. A count is noted when rat cuts off an infrared beam falling on the photocell.

The calculation of locomotor activity of CPZ induced rats was done withactophotometer. The locomotor activity was noted as the number of counts 5 min per rat.[79]


**Figure 6: recording the locomotor activity using Actophotometer.**

## DISSECTION AND HOMOGENISATION

After performing experimental treatment of 21 days, the rats were given overdose of Anesthetic ether and sacrificed. The brains of the experimental rats were carefully removed placing the cerebellum and separating out the forebrains. The weights of brains of the rats were noted and 10% v/w homogenates were prepared in phosphate buffer (0.1M, pH 8).

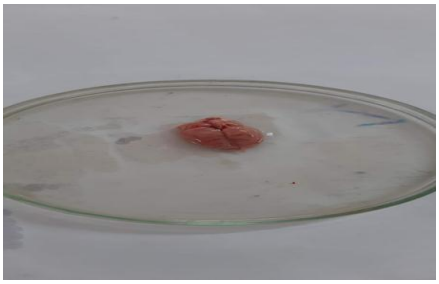

**Figure 7: dissected rat brain.**

**Biochemical Estimation**

**1.Estimation of Dopamine**

**Principle:** DA is a regulatory neurotransmitter fundamental for the movement and cognition. Since PD is characterized by the loss of the DAnergic neurons, the neuronal damaged brain is differentiated with low levels of DA.

**Reagents Used**

- Hcl-Butanol (1:10)
- 0.1 M HCl
- Hexane
- 0.4M HCl
- Buffer- Sodium acetate (pH 6.9)
- Iodine solution (0.1M in ethanol)
- Acetic acid
- Sodium sulphite solution

**Procedure**

The tissue of rat brain was homogenized in Hcl-butanol for one minute after being weighed, then centrifuged at 3000rpm for 10 minutes. To the separator tube having 0.1MHydrochloricacid and hexane (2.5ml), aliquot supernatant was added. The assay was carried out by utilizing an aqueous phase (0.2ml) at 0°C. To the aqueous phase (0.2ml), 0.4M Hcl(0.05ml) & Sodium acetate buffer, pH 6.9 subsequent to 0.1M in ethanol-Iodine solution (0.1ml) for oxidation. Sodium sulphite solution (0.1ml) was added after 2 minutes for stopping, the adding of acetic acid (0.1ml) after 90 seconds. Solutions were heated to 100°C for few minutes. The radiation and excitation spectra were noted at 330375nm from the spectrofluorimeter. With the addition oxidation reagents in opposite order, (iodine after sodium sulphite) then the tissue blanks were set.[80]

$$X_{Dopamine} = \frac{\text{Sample OD} - \text{Blank OD}}{\text{Standard OD} - \text{Blank OD}} \times \text{Concentration of Standard}$$

**2. Lipid peroxidation Estimation**

**Principle**

Thiobarbituric acid reactive substances (TBARS) in tissues is a metric for lipid peroxidation. Thiobarbituric acid responsive product is formed when

malondialdehyde (MDA) engaegesthiobarbituric acid at 95° for 30 minutes in acidic medium. A pink coloured product is formed and absorbance of its can be measured at 532 nm.

**Reagents Used**
- Thiobarbituric acid with distilled water
- SLS solution
- n-butanol and pyridine mixture (15:1 v/v)
- Acetic acid with distilled water

**Procedure**
To 5ml solution was made use of 1ml of homogenate, 0.2 ml SLS, 1.5ml each of acetic-acid and TBA and incubated for few minutes and then heated for 30 minutes in a water bath. N-butanol- pyridine mixture was employed to extract the chromagen and centrifugation is done for 10 min at 4000 rpm. At 532 nm, the absorbance of the organic coating was evaluated. The concentration of MDA is stated as Nano moles/mg of protein.[81]

$$\text{Conc. Of MAD} = \frac{Abs_{532} \times 100 \times V_T}{(1.56 \times 10^5) \times W_T \times V_U}$$

Where,
Wt = weight of brain after dissection
Vu = volume of aliquot
Vt = volume in a cuvette,

**3. Glutathione Estimation**
**Principle**
5, 5 – dithio – bis – (2, nitrobenzoic acid) i.e DTNB reagent used, that is a disulphidechromagen that is readily condensed by GSH to form a product of yellow colour . The absorbance is equivalent to glutathione which was measured at 412nm

**Reagents Used**
- 20% trichloroacetic acid
- Ellmans reagent (0.1mM)
- 1mM EDTA
- 1% sodium citrate solution
- 0.3M phosphate buffer

**Procedure**
To homogenate of rat tissue, 20% trichloroacetic acid in equivalent vol. containing 1mM EDTA, for a few minutes. It was centrifuged (200rpm) for 10 minutes and 200µl of supernatant was taken. 1.8ml of Ellman's reagent (0.1mM) was made with 1% sodium citrate solution, 0.3M phosphate-buffer. The solution was measured at 412nm.[82]

**Calculations**

$$\text{GSH level} = \frac{Y - 0.00314}{0.0314} \times \frac{D_F}{B_T \times V_U}$$

Where,
Y – $Abs_{412}$
$D_F$ – Dilution factor (1)
$B_T$ – Brain tissue homogenate (1ml)
$V_U$ – Volume (1ml)

**4. Superoxide Dismutase Estimation**
**Principle**
Superoxide Dismutase (SOD) follows the reaction, in which it catalyzes the superoxide (O2-) to hydrogen peroxide (H2O2) and molecular oxygen(O2).

**Reagents**
- 0.05M Carbonate bicarbonate buffer
- 0.1N HCl (pH 2)
- Epinephrine (3 x 10-2M)

**Procedure**
To the bicarbonate carbonate buffer (pH 9.7) the upper layer (0.1ml) was added. To this, epinephrine was taken slowly then absorbance estimated at 480nm for 2 mins. 129
Calculation:

$$\text{SOD (Enzyme units)} = \frac{MB - MT}{MB \times 50} \times 100$$

Where,
MB = Mean absorbance of blank/ minute
MT = Mean absorbance of test/ minute

**RESULTS**

**Statistical Analysis**
Values were expressed as mean ± SEM (where, n=6). Statistical evaluation of the data was performed by one–way ANOVA followed by Dunnett's multiple comparison test by using **Graphpad Prism 8.4.3 Software.**

Results were compared to be statistically significant at *$p \leq 0.05$.

**Preliminary phytochemical analysis**

**Table 2: Phytochemical investigations of MEPAC showed the following phytoconstituents.**

| S.NO | TESTS | METHANOLIC EXTRACT |
|---|---|---|
| 1. | ***Tests for Carbohydrates*** | |
| | Benedict's test | + |
| | Molisch's test | + |
| | Fehling's test | + |
| 2 | ***Test for Flavonoids*** | |
| | Shinoda test<br>Lead acetate test | + |
| 3 | ***Tests for Phenols and Tannins*** | |
| | $FeCl_3$ Solution test | - |
| | Acetic acid | - |
| | $HNO_3$ test | + |
| 4 | ***Test for Proteins*** | |
| | Biuret test | + |
| | Xanthoproteic test | - |
| | Millions test | - |
| 6 | ***Tests for Alkaloids*** | |
| | Dragendorff's test | + |
| | Mayer's test | + |
| | Tannic acid test | + |
| 7 | ***Test for Saponins*** | |
| | Foam test | + |
| 8. | **Test for steroids** | |
| | Salkowski test | + |
| | Liebermann Burchard test | + |

**Behavioural Assessments**

**a. Locomotor activity (Actophotometer)**

**Table 3: Effect of MEPAC on Locomotor activity of experimental PD rats.**

| | Locomotive Score | | | |
|---|---|---|---|---|
| **GROUPS** | **Day 1** | **Day 7** | **Day 14** | **Day 21** |
| **Control** | 184.8 ± 5.54**** | 183 ±3.07**** | 189 ± 2.38**** | 186.7 ± 2.79**** |
| **Disease Control** | 74.17 ± 5.38$^{\#\#}$ | 70 ± 6.19$^{\#\#\#}$ | 74.7 ± 3.52$^{\#\#\#\#}$ | 76.67 ± 4.41$^{\#\#\#\#}$ |
| **Standard** | 173.3 ± 3.33**** | 172.8 ± 5.12**** | 173.8 ±4.206**** | 171.7 ± 3.07**** |
| **PAME (200mg/kg)** | 81.2 ± 4.13$^{ns}$ | 87.5 ± 5.28$^{ns}$ | 96.5 ± 4.072** | 122.5 ± 4.95** |
| **PAME (400mg/kg)** | 129.8 ±3.42$^{***}$ | 152.2 ± 4.28*** | 152.5 ± 5.28**** | 152.5 ± 3.59**** |

Each value mentioned here is expressed as Mean ± SEM (where n = 6, each group). ANOVA-I was performed using Dunnett's multiple comparison test. The probability- ***P < 0.001, **P< 0.001 in contrast with vehicle + control group and ns – not significant.

The syndopa group produced an increase in dose dependent activity through the experimental treatment. PAME (400mg/kg) exhibited its activity very close to the standard group with significant results.

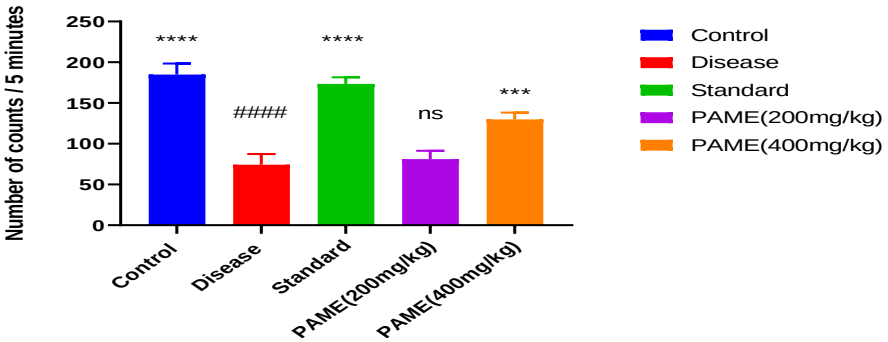


**Figure 8: LOCOMOTOR ACTIVITY ON DAY1.**

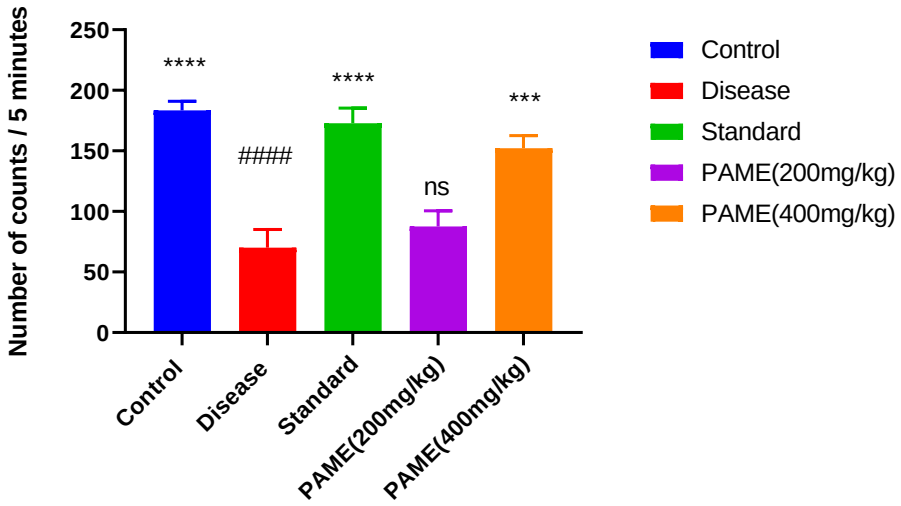


**Figure 9: LOCOMOTOR ACTIVITY ON DAY7.**

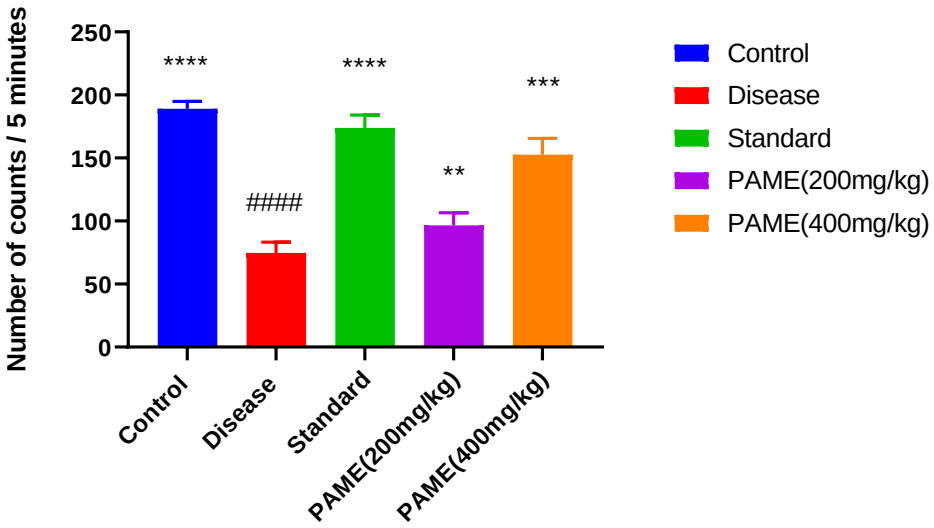


**Figure 10: LOCOMOTOR ACTIVITY ON DAY14.**

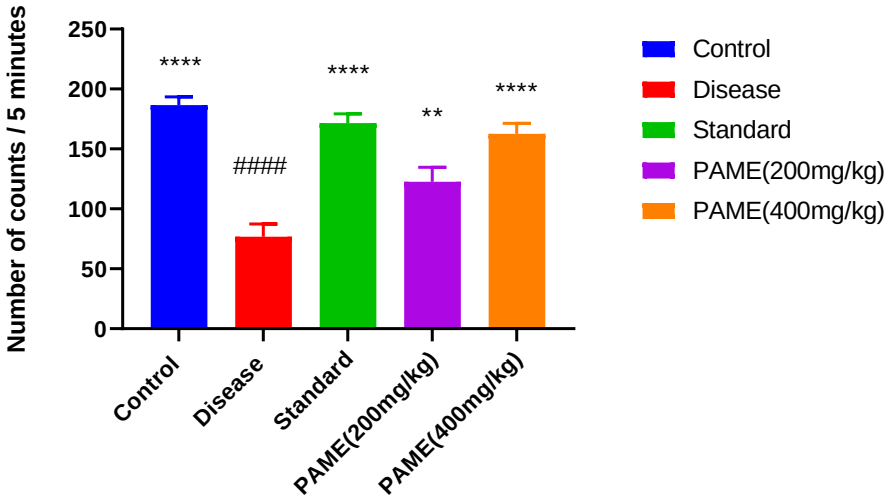


**Figure 11: LOCOMOTOR ACTIVITY ON DAY 21.**

**B. Catalepsy (Block method)**

**Table 4: Effect of MEPAC on Cataleptic behaviour of experimental PD rats.**

| | Cataleptic Score | | | |
|---|---|---|---|---|
| **GROUPS** | **Day 1** | **Day 7** | **Day 14** | **Day 21** |
| **Control** | 0 | 0 | 0 | 0 |
| **Disease Control** | 2.91 ±0.1$^{####}$ | 2.98 ± 0.08$^{####}$ | 2.98 ±0.09$^{####}$ | 3.07 ± 0.08$^{####}$ |
| **Standard** | 1.25 ±0.07**** | 1.38 ± 0.05**** | 1.8 ± 0.036**** | 1.47 ± 0.04**** |
| **PAME (200mg/kg)** | 2.78 ± 0.07$^{ns}$ | 2.88 ± 0.07 $^{ns}$ | 2.86 ± 0.09 $^{ns}$ | 2.03 ± 0.17** |
| **PAME (400mg/kg)** | 1.36 ± 0.08*** | 1.40 ± 0.07*** | 1.45 ± 0.04**** | 1.36 ± 0.04**** |

Each value mentioned here is expressed as Mean ± SEM (where n = 6, each group). ANOVA-I was performed using Dunnett's multiple comparison test. The probability- ***P < 0.001, **P< 0.001 in contrast with vehicle + control group and ns – not significant.

PAME group animals prompted a fall in the catalepsy in contrast with the disease group. The standard group showed a decrease in dose-dependent throughout the experimental treatment. PAME (400mg/kg) exhibited its activity very close to the standard group with significant results.

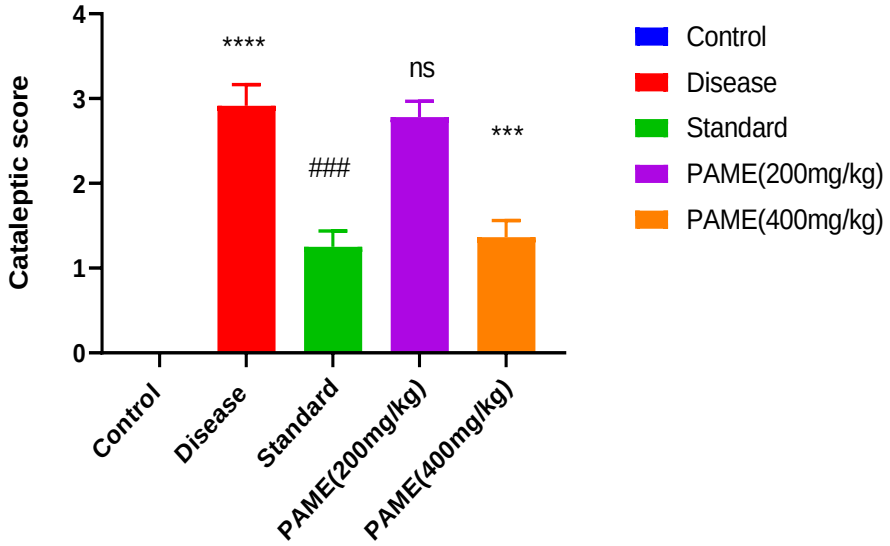


**Figure 12: Cataleptic Score day 1.**

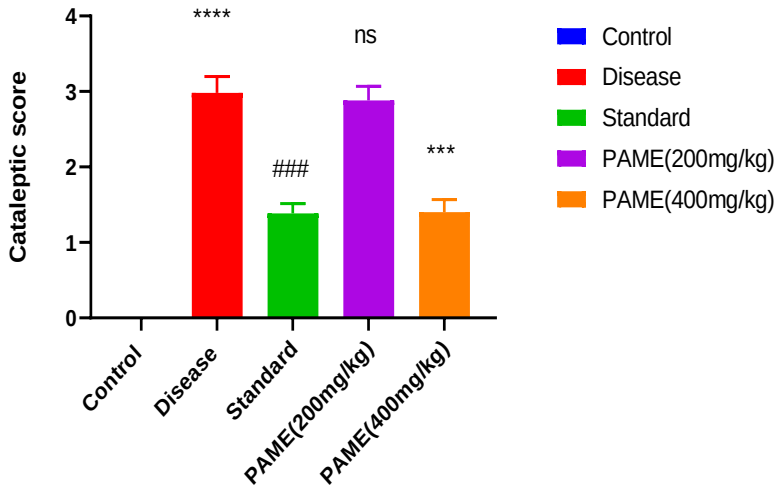


**Figure 13: Cataleptic Score day 7.**

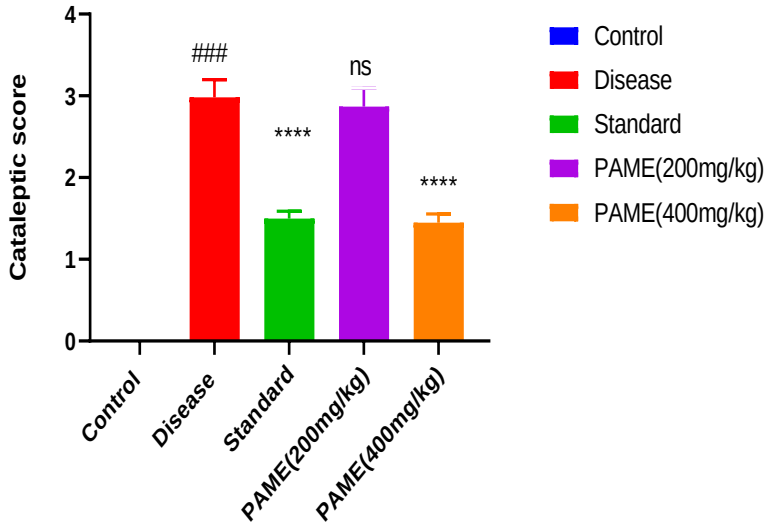


**Figure 14: Cataleptic Score day 14.**

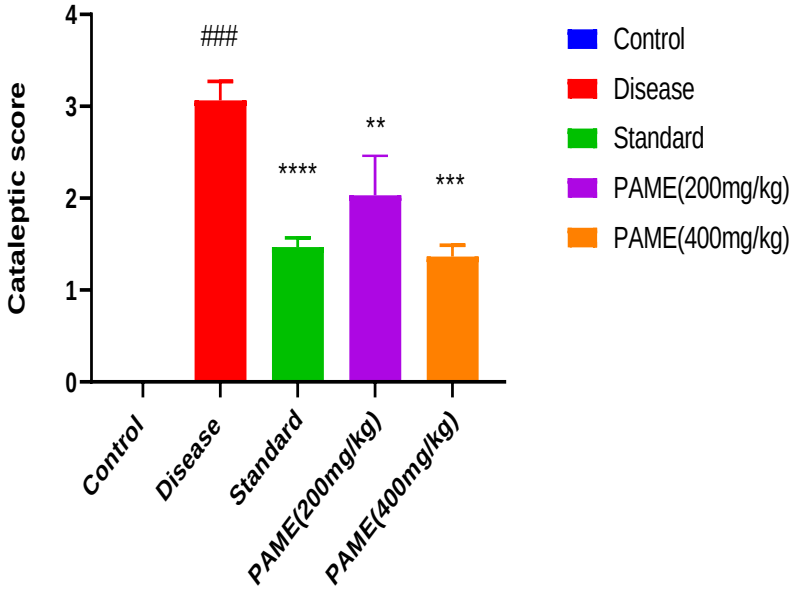


**Figure 15: Cataleptic Score day 21.**

**Biochemical Estimation**
**Dopamine estimation**
**Table 5: Effect of PAMA on the levels of DA in rat brain.**

| TREATMENT GROUPS | MEAN ± SEM |
|---|---|
| **Control** | 0.60 ± 0.05$^{****}$ |
| **Disease Control** | 0.27 ± 0.02$^{####}$ |
| **Standard** | 0.57 ± 0.01$^{****}$ |
| **PAME(200mg/kg)** | 0.35 ± 0.01$^{**}$ |
| **PAME(400mg/kg)** | 0.50 ± 0.08$^{***}$ |

Each value mentioned above as Mean ± SEM (where n = 6 in the individual group). ANOVA-I along with Dunnett's multiple comparison test was performed. ***P < 0.001, **P< 0.001 was found when compared with control group ns – not significant.

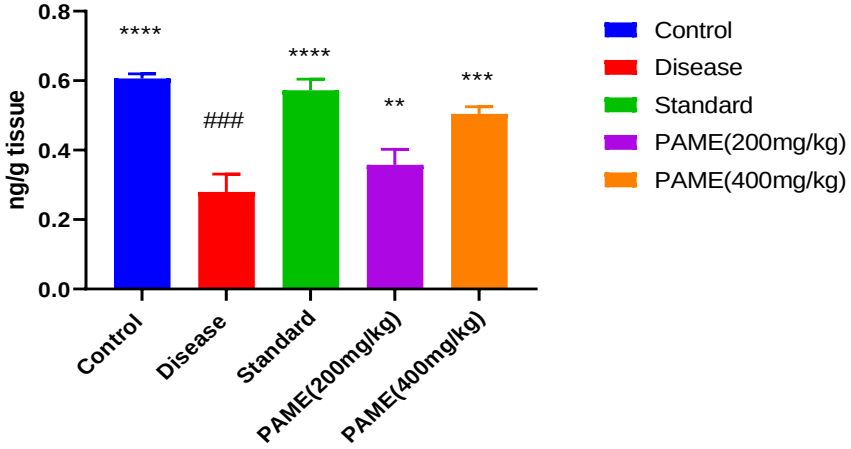


**Figure 16: Graph represent the effect ofPAMEon dopamine level.**

**Lipid Peroxidation Estimation**

**Table 6: Result of PAME on the levels of MDA in rat brain.**

| TREATMENT GROUPS | MEAN ± SEM |
|---|---|
| **Control** | 0.60 ± 0.08**** |
| **Disease Control** | 0.32 ± 0.02### |
| **Standard** | 0.56 ± 0.01**** |
| **PAME(200mg/kg)** | 0.45 ± 0.06*** |
| **PAME(400mg/kg)** | 0.50 ± 0.12**** |

Each of the value stated above as Mean ± SEM (where n = 6 in the individual group). ANOVA-I along with Dunnett's multiple comparison test was performed. ***P < 0.001, **P< 0.001 and *P< 0.05 was found when related with standard drug i.e., syndopa induced disease group. ###P < 0.001 found when compared with control group ns – not significant.

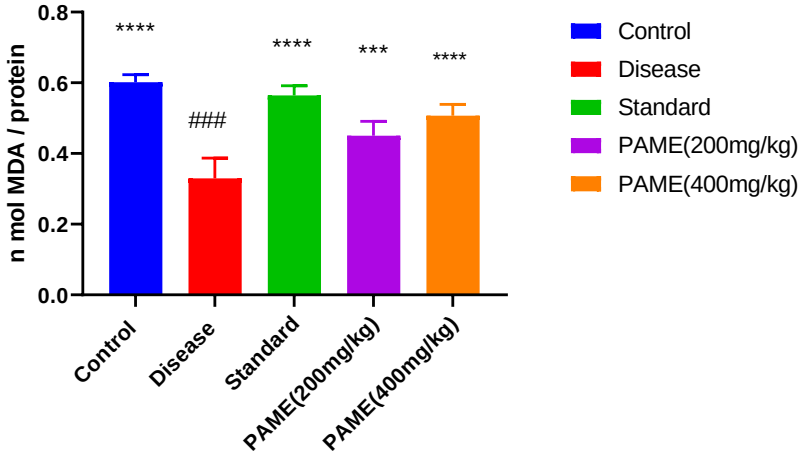


**Figure 17: Graph represent the effect of PAMA on MDA level.**

**Reduced Glutathione Estimation**

**Table 7: Results of PAME on the levels of GSH in rat brain.**

| TREATMENT GROUPS | MEAN ± SEM |
|---|---|
| **Control** | 0.58 ± 0.09**** |
| **Disease Control** | 0.35 ± 0.01#### |
| **Standard** | 0.52 ± 0.01**** |
| **PAME(200mg/kg)** | 0.42 ± 0.01**** |
| **PAME(400mg/kg)** | 0.47 ± 0.07**** |

Each value stated above as Mean ± SEM (where n = 6 in the individual group). ANOVA-I with Dunnett's multiple comparison test was performed. ***P < 0.001, **P< 0.001 and *P< 0.05 was found when associated with standard drug i., syndopa induced disease group. ###P < 0.001 found when compared with control groups – not significant.

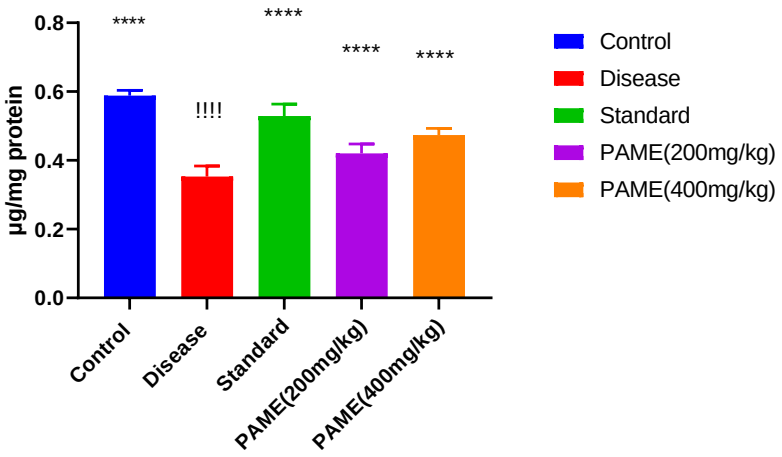


**Figure 18 Graph represent the effect of PAMA on GHS level.**

**Superoxide Dismutase Estimation**

**Table 8: Effect of MEPAC on the levels of SOD in rat brain.**

| TREATMENT GROUPS | MEAN ± SEM |
|---|---|
| **Control** | 0.61± 0.01**** |
| **Disease Control** | 0.31 ± 0.07### |
| **Standard** | 0.55 ± 0.01**** |
| **PAME(200mg/kg)** | 0.41 ± 0.02** |
| **PAME(400mg/kg)** | 0.48 ± 0.09*** |

Each value stated above as Mean ± SEM (where n = 6 in the individual group). ANOVA-I along with Dunnett's multiple comparison test was performed. ***P < 0.001, **P< 0.001 and *P< 0.05 was found when compared with standard drug i.e., syndopa induced disease group. ###P < 0.001 found when compared with the control group's – not significant.

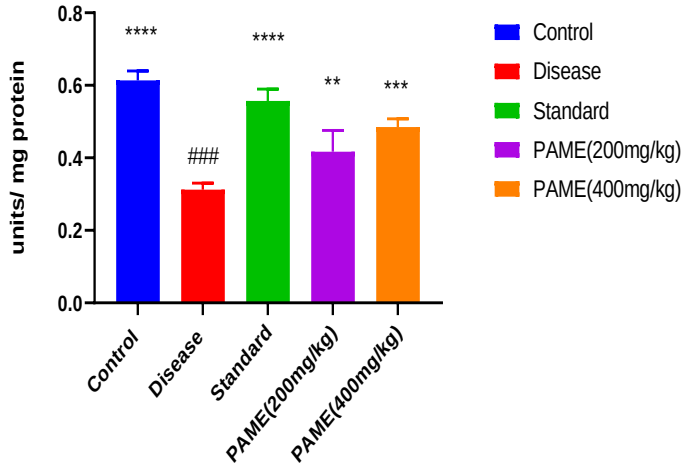


**Figure 19: graph represent the effect of PAMA on SOD level.**

**Histopathological Evaluation**

The brains of rats were dissected and were transferred into a container containing 10% formalin solution and were used for conducting a histopathological evaluation.

**Histopathology**

The results of the histopathology of brains of rats were described as follows.

**1. Normal control brain**

The group of these rats showed no sign of inflammation & no injury. Meninges which surround the cortex appeared to be normal. Hippocampus of the brain showed no signs of any damage or pathological change hence appeared normal.(Figure A,B,C).

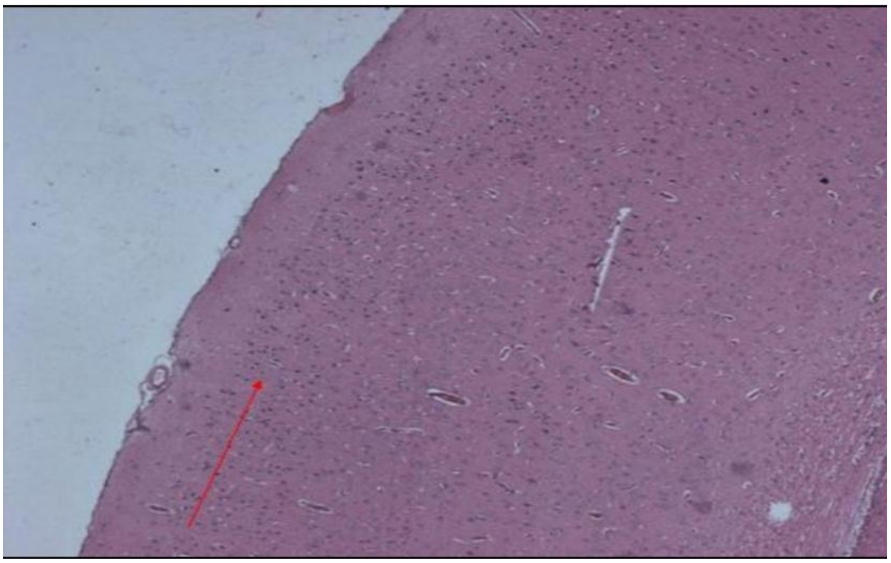

**Figure 20 (A): The arrow point to the cerebral cortex of rats of Normal group.**

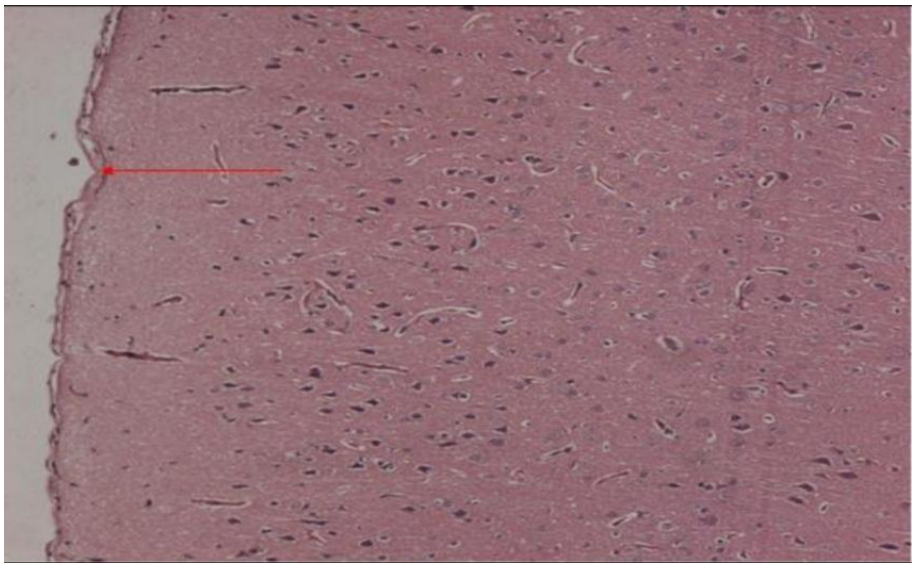

**Figure 21(B): The arrow indicates the Meninges surrounding cerebral cortex of the rats of Normal group.**

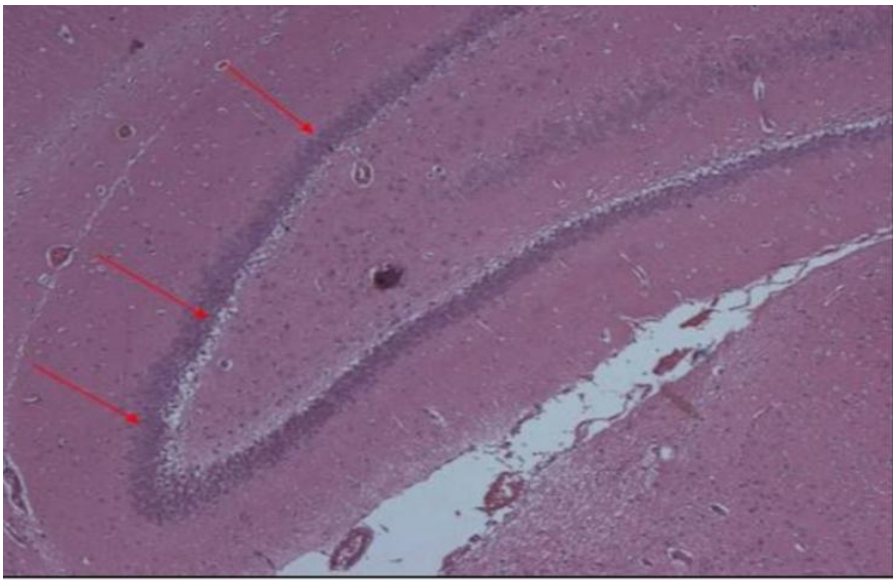

**Figure 22 (C): The arrow indicates the Hippocampus region of rats of Normal group.**

**2. Diseased brain**

In group of these rats Multi focal necrosis was observed in the cerebral cortex of brain and mild proliferation of neurons was observed. In the meningeas of brain foci of meningitis was observed.(Figure A,B,C).

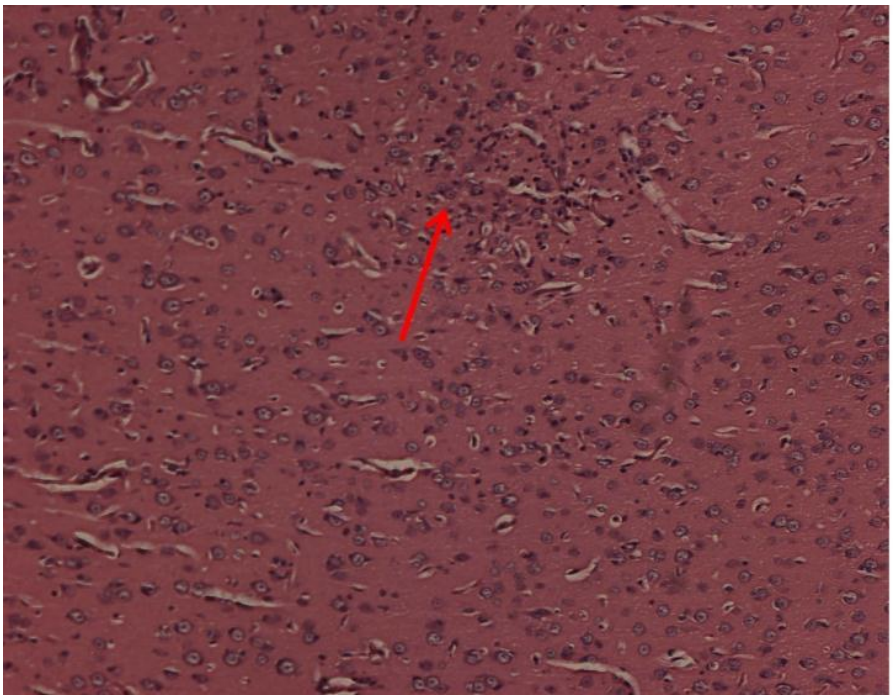

**Figure 23: (A) The Arrow represents Multifocal necrosis in the cerebral cortex of the brain.**

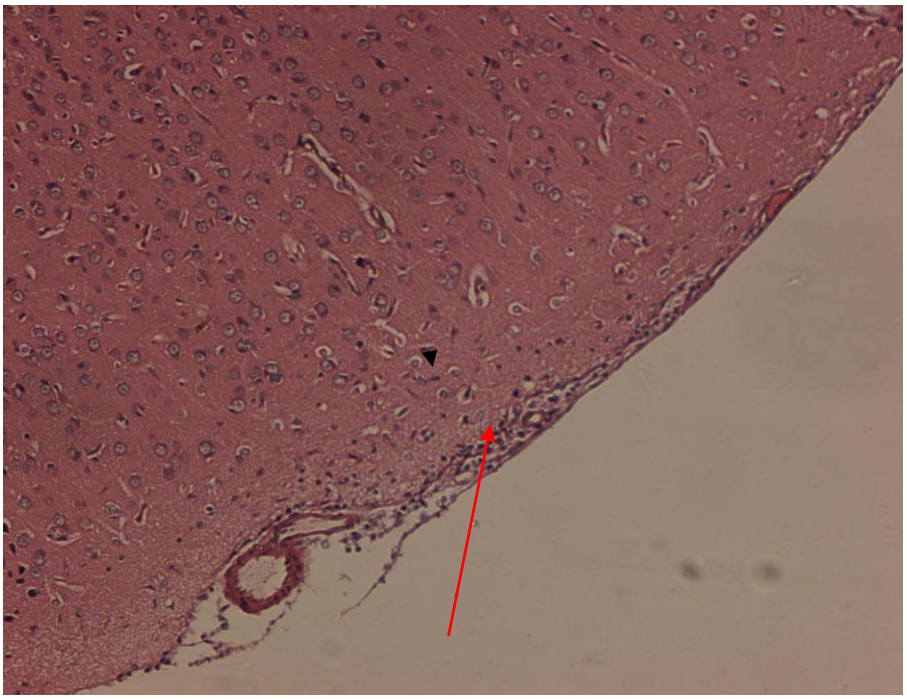

**Figure 24: (B) The Arrow represents Foci of meningitis in the meningeas of the brain.**

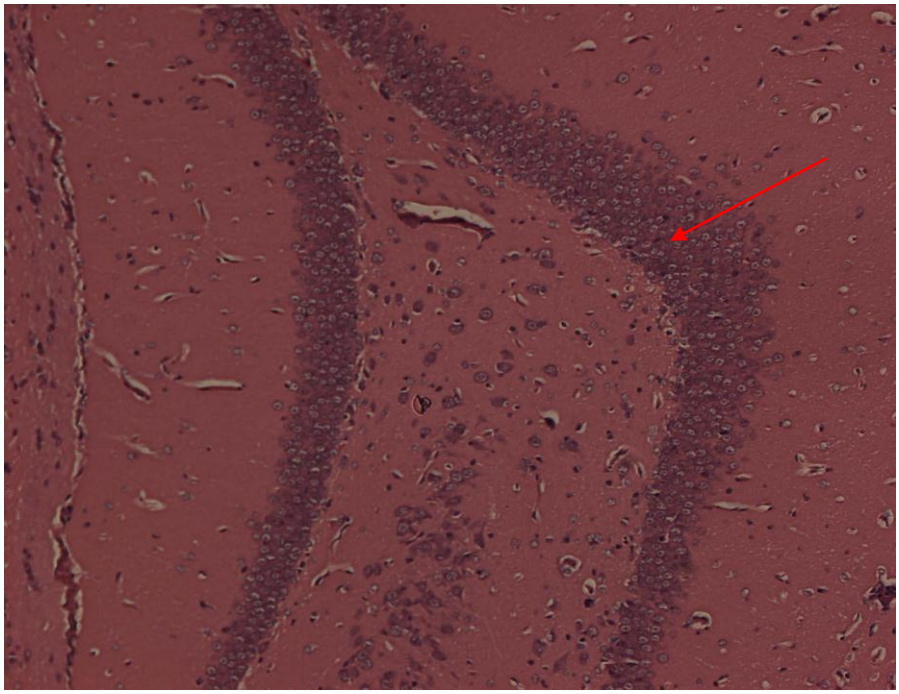

**Figure 25: (C) The Arrow represents Mild proliferation of neurons in the disease control group.**

**3. Standard brain**

In this group of rats showed a normal cerebral cortex and hippocampus. It also shownfoci of meningitis in the meninges covering cerebral hemisphere. Figure (A,B,C).

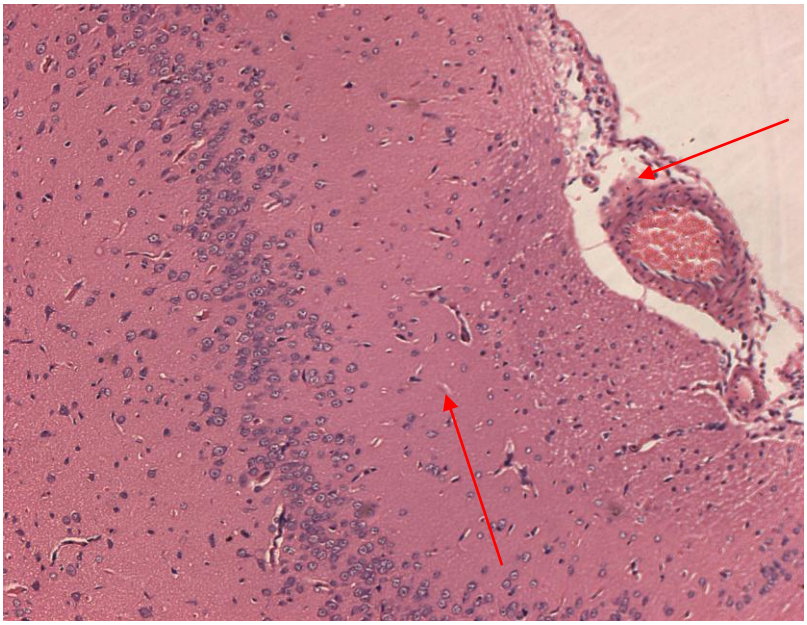

**Figure 26: (A) The Arrow represents normal cerebral cortex and normal meningeas.**

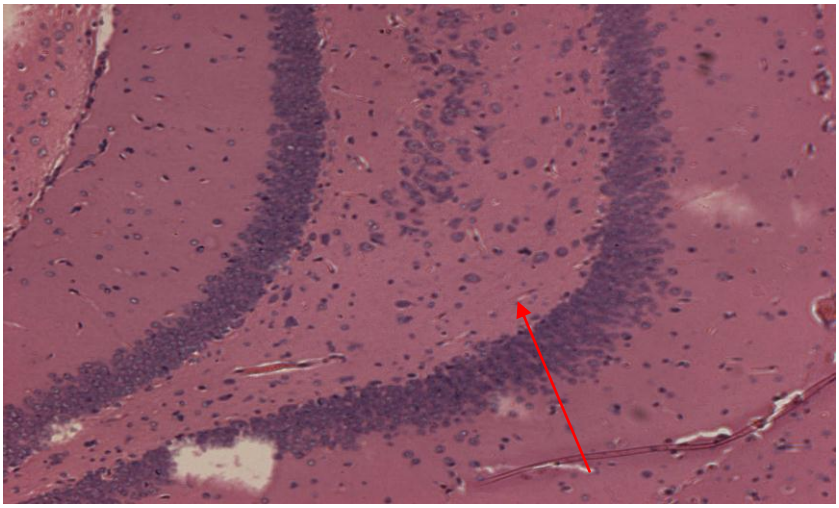

**Figure 27: (B) The Arrow represents normal Hippocampus region of the standard group.**

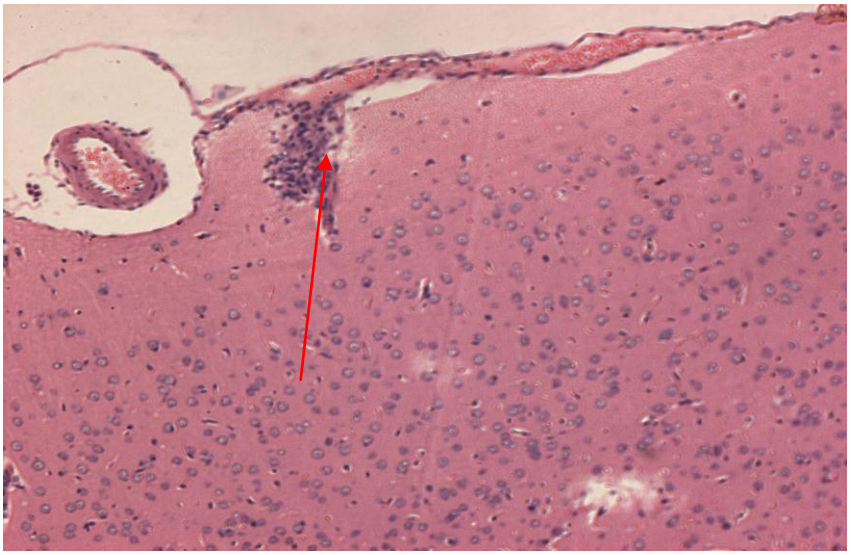

**Figure 28: (C) The arrow indicates Foci of meningitis in the meninges covering cerebral hemisphere.**

**4.Treatment group I(PAME 200mg/kg)**

In this group of rats showed normal cerebral cortex with normal meninges covering the cerebral hemisphere. The hippocampus of the brain also seemed normal with the mild proliferation of neurons. Figure(A,B,C).

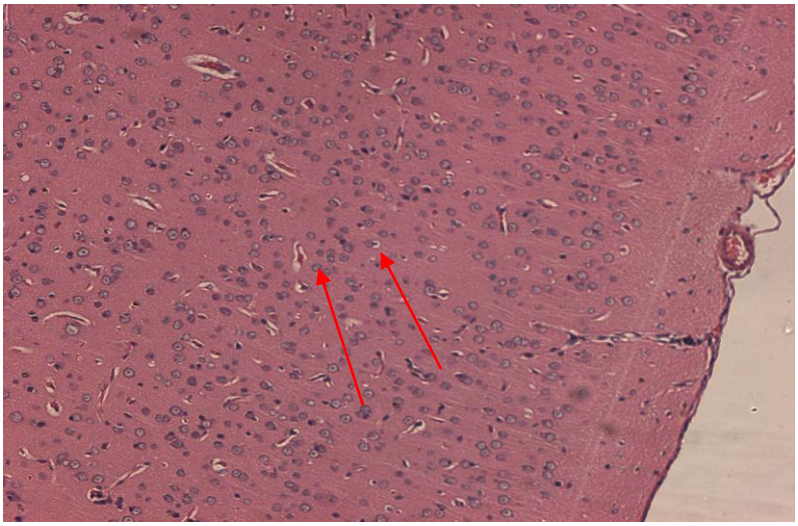

**Figure 29: (A) The Arrow directs the normal cerebral cortex.**

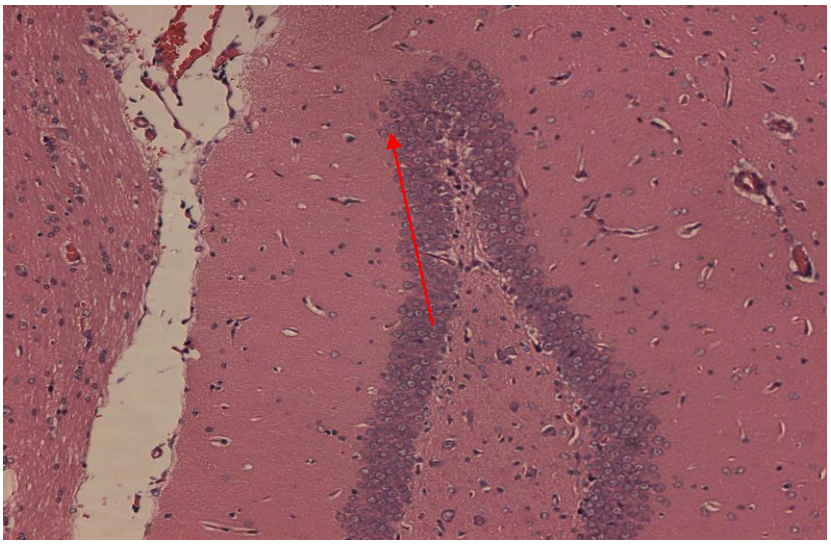

**Figure 30: (B) The Arrows indicates the normal hippocampus region with a mild proliferation of neurons.**

**5.Treatment group II(PAME 400mg/kg)**

In this group of rats indicated the presence of normal cerebral cortex with meninges covering the cerebral hemisphere. Hippocampus region of the brain also appeared normal with apoptotic neurons was observed in few places.Figure (A,B,C).

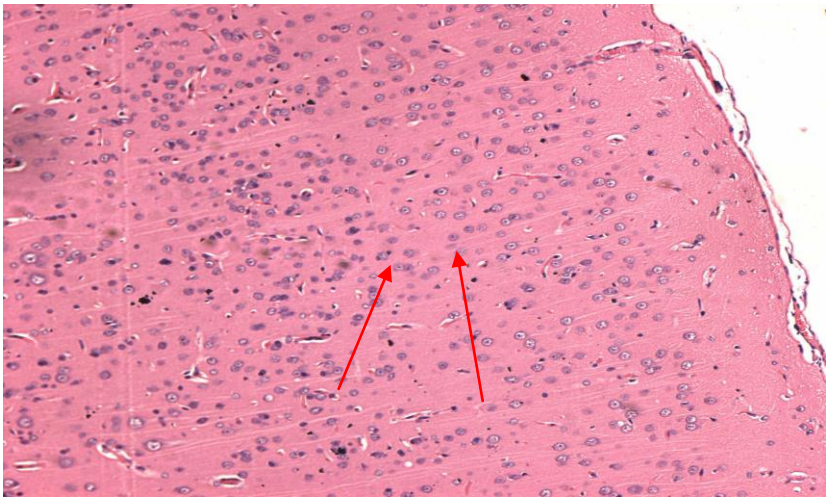

**Figure 31: (A) The Arrow shows the normal cerebral cortex.**

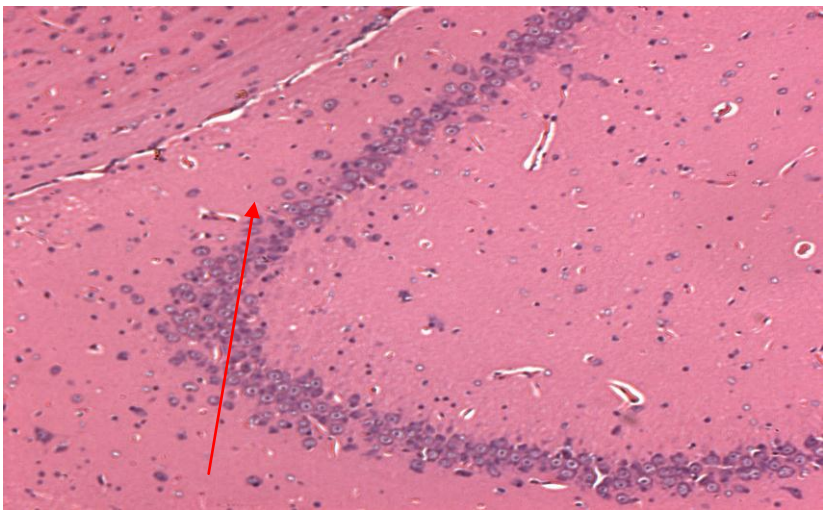

**Figure 32: (B): The Arrow shows the normal hippocampus.**

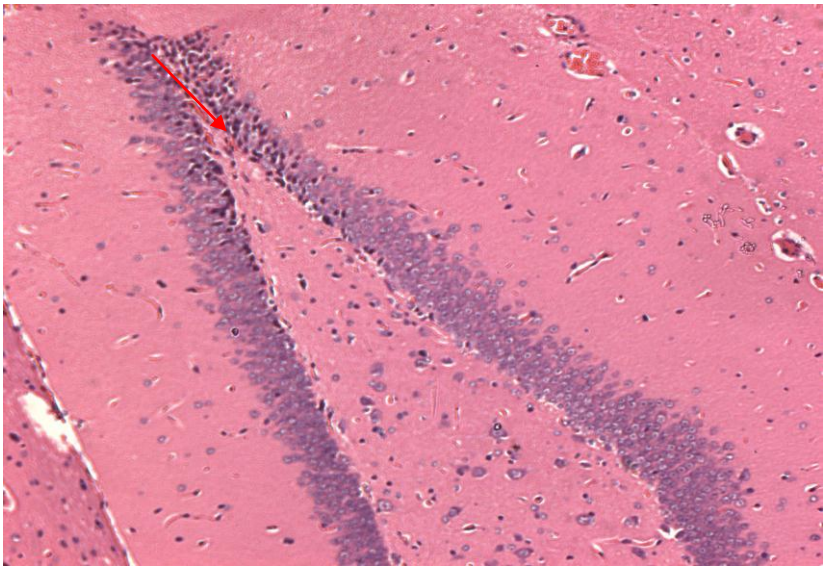

**Figure 33(C): The arrow indicates normal Hippocampus, but apoptotic neurons were observed in a few places.**

## DISCUSSION

Parkinson's disease is a neurodegenerative disorder influencing millions and has accounts of its depictions since earliest times. Mainly a disease of the elderly found to impact younger populaces in some cases. The primary pathology of this is the harm dopaminergic neurons in the SNpc of the midbrain. The Parkinson disease patients are associated with resting tremors, rigidity, bradykinesia and postural instability. There is no complete cure for Parkinsons disease and the present-day medicines are generally relied on up-on to give symptomatic relief and refine personal satisfaction. levodopa and DA agonists endure pharmacological treatment alternatives and surgical treatment has opted in serious cases. However, the medications utilized in PD also have various adverse effects.

In the present study, the leaves of phyllanthusacidus were used to find its neuroprotective effect in chlorpromazine induced Parkinson animal model. Phyllanthusacidus has chemical constituents such as quercetin, gallic acid, kaempferol and its also rich source of secondary metabolites and various alkaloids, saponins, terpenes and anthraquinones. Products obtained from natural plants are well tolerated and also associated with fewer side effects when matched with synthetic drugs.

In this study, chlorpromazine was used to induce Parkinson's in albino rats. Chlorpromazine is an antipsychotic drug which shows extrapyramidal side effect as it's a dopamine receptor blocking agent.

The study was executed for 21 days. Rats were divided into 5 groups, standard drug syndopa(10mg/kg) was used and PAME (200mg/kg and 400mg/kg) was used as a test drug.

During 21 days behavioural parameters such as catalepsy, locomotor activity and muscle coordination were performed. After 21 days, rats were dissected and their brains were removed then homogenized and centrifuged for the biochemical estimation such as dopamine, SOD, GSH and catalase.

The catalepsy in the rats was assessed using a wooden block method. The cataleptic score was assigned to each group. The group treated with CPZ showed a protective effect i.e., reduced cataleptic score.

Locomotor activity was measured using an actophotometer indicates that CPZ significantly reduces locomotor counts. Syndopa(10mg/kg) and PAME(400mg/kg) significantly increased the locomotor activity as compared to CPZ treated animals.

As one of the symptoms of PD is a motor imbalance to evaluate this symptom Rotarod test was performed. The results showed that rats treated with syndopa(10mg/kg) & PAME(200mg/kg& 400mg/kg) shown an improved motor movements.

DA is a neurotransmitter that plays a vital role in motor control and movement. Neuronal damaged-brain manifest stunted levels of DA.134,5 1In the present study, On treatment with PAME (200mg/kg) and PAME (400mg/kg), the DA levels were (0.35 ± 0.01) and (0.50 ± 0.08) in vicinity of Syndopa (0.57 ± 0.01).

LPO is considered as bio-marker and involved in PD pathogenesis.137 PAME (200mg/kg) and PAME (400mg/kg), reduced LPO (0.45 ± 0.06) and (0.50 ± 0.12) which was proximal to Syndopa group (0.56 ± 0.01).

The GSH when diminished, ROS become prone since defence against them is hindered.139.In this study, On treatment with PAME (200mg/kg) and PAME (400mg/kg), the GSH levels were (0.42 ± 0.01) and (0.47± 0.07) in similarity with Syndopa (0.52 ± 0.01).

SOD is an anti-oxidart enzyme protecting ROS., On treatment with PAME (200mg/kg) and PAME (400mg/kg), the DA levels were 0.41 ± 0.02) and(0.48 ± 0.09) in vicinity of Syndopa (0.55 ± 0.01).

Histopathological reports showed that the group treated with Syndopa (10mg/kg), PAME (200mg/kg & 400mg/kg) exhibit a protective effect when compared to the disease control group by not indicating any signs of inflammation, mild apoptosis also exhibited a mild proliferation in the region of the hippocampus

## CONCLUSION

The methanolic leaf extract of *phyllanthus acidus* was selected to study the anti-parkinsonian activity in chlorpromazine induced Parkinson animal model in Wistar albino rats.

The behavioral assessment indicates that PAME treated rats shown a decrease in its cataleptic score and rise in locomotor activity while comparing that with the diseased treated group.

The rats treated with PAME shown an increasing level of DA, GSH, SOD contrast to CPZ treated groups. On the other side, the group treated with syndopa 10mg/kg, PAME 200mg/kg and PAME 400mg/kg showed a neuroprotective activity by improving cataleptic behavior, motor movement and locomotors activity.

The reports of histopathology also suggest that PAME treated rats indicated results similar to that of the standard group.

Thus, the final conclusion is that the methanolic extract of phyllanthusacidus showed significant anti-parkinsonian activity and antioxidant activity.

However, additional extensive studies are required to examine the phytochemical constituents responsible for the detailed MOA for the prevention and treatment of PD.