

EFFECT OF *PHYLLANTHUS AMARUS* ON HALOPERIDOL INDUCED CATALEPSY IN  
EXPERIMENTAL ANIMAL MODELSDeepa B<sup>1</sup>, Anu E Joy<sup>2</sup> and Shyamjith Manikkoth<sup>3\*</sup><sup>1</sup> Department of Pharmacology, K.S.Hegde Medical Academy, Nitte University, Mangalore<sup>2</sup> Department of Physiology, Yenepoya Medical College, Yenepoya University, Mangalore<sup>3</sup> Department of Pharmacology, Yenepoya Medical College, Yenepoya University, MangaloreCorresponding Author\*: Email: [shyamjithpubs@gmail.com](mailto:shyamjithpubs@gmail.com) Mobile: +919480361961**ABSTRACT**

**Objectives:** The aim of the study was to investigate the anticataleptic effect of *Phyllanthus amarus* ethanolic extract in Swiss albino mice.

**Methods:** The ethanolic extract of leaves of *Phyllanthus amarus* [PAEE] at a dose of 100mg/kg/body weight was administered orally for ten days. On tenth day, one hour later Haloperidol [1 mg/ kg IP] was administered to induce catalepsy.

**Results:** The results indicate that induction of catalepsy by Haloperidol in Swiss albino mice was significantly prevented by PAEE.

**Conclusions:** The anticataleptic activity of *Phyllanthus amarus* can be due to its effect on brain neurotransmitters or due to antioxidant property.

**Key Words:** Haloperidol, catalepsy, Swiss albino mice, PAEE, Anticataleptic

**INTRODUCTION**

Parkinson's disease, first described by James Parkinson in 1817, is a neurodegenerative ailment resulting from the damage of nerve cells in the brain. It's the second most common age-related neurodegenerative disease after Alzheimer's disease. The condition mainly affects movement, especially in the early stages. As this disease progresses, various parts of the nervous system may be affected, resulting in depression, cognitive impairment and physical conditions such as bladder problems. It is a progressive illness with a mean age onset at 55, and the prevalence increases significantly with age. The etiology of Parkinsonism is unidentified, with uncertainty about the role of environmental toxins and genetic aspects. It can be also caused by medications (Joshi SV et al, 2011; Hae-Won Shin et al, 2012; William Dauer et al, 2003).

The commonly used antiparkinsonian drug like L-Dopa mainly replenishes dopamine levels or the drug like pramipexole mimics the action of dopamine in CNS. In the event of drug induced Parkinsonism, the commonly used drugs are centrally acting anticholinergics like benzhexol to counter the excessive over activity of acetylcholine in substantia nigra pars compacta. Even though they are highly effective in alleviating the symptoms of Parkinsonism, these drugs do not give complete cure. Moreover, these drugs are often associated with frequent side effects (Bhushan Gandhare, 2012; Vinod Nair et al, 2007; William Dauer et al, 2003). The significance of many indigenous medicinal plants and their phytoconstituents in the management of Parkinsonism with minimal side effect profile arise in this context. Haloperidol produces catalepsy i.e., a state of akinesia with muscular rigidity in animals. It is an established model for screening the drugs for anti-parkinsonian effect (Bhushan Gandhare, 2012; Ranjita B et al, 2002; Vinod Nair et al, 2007). *Phyllanthus amarus* (Syn. *Phyllanthus niruri*) is a perennial herb generally seen in the tropical and subtropical regions of both hemispheres. It belongs to Euphorbiaceae family. *Phyllanthus amarus* is widely used all over the world for treating ailments like jaundice, asthma, hepatitis, urogenital problems, dysentery, dyspepsia, arthritis, malaria, etc.

Numerous pre-clinical studies proved that this plant possess antiviral, analgesic, anticonvulsant, antioxidant, antimicrobial, anti-amnesic, antitumour, hepatoprotective, hypoglycaemic and hypolipidemic activities (Aruna Kumar R et al, 2010; Bhattacharyya, 2003; Bhattacharjee R, 2007; Hanumanthachar Joshi et al 2007; Priyanka Sharma, 2009; Shetti AA et al, 2012; Rashmi Mathur et al, 2011; Rajeshwar Y et al, 2008; Shyamjith M et al, 2011; Sundeep Hegde et al, 2014).

This research work was conducted to evaluate the effect of ethanolic extract of *Phyllanthus amarus* leaves in the treatment of Haloperidol induced catalepsy, which is an established model for screening antiparkinsonian drugs.

## MATERIALS AND METHODS

### Drugs and Chemicals

Haloperidol (Serenace, R.P.G Life Science Ltd, Ankleshwar.) was obtained from a pharmacy in Mangalore. It was administered at a dose of 1 mg/ kg IntraPeritoneally [I.P]. The Standard drug Scopolamine (Buscopan, Cadilla Health Care Ltd, Goa.) was also obtained from a pharmacy in Mangalore. It was administered at a dose of 1 mg/ kg I.P.

### Plant materials

*Phyllanthus amarus* were cultivated during the month of June. The fresh leaves were collected in the month of September. They were authenticated by Dr.Noeline J.Pinto, Head of Botany department, St.Agnes College, Mangalore, Karnataka, India.

They were shade dried, and then powdered.

### Sample Preparation and Extraction

*Phyllanthus amarus* ethanolic extract (PAEE): A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60°C. The percentage yield of the extract was 20%. The ethanolic extract was dissolved in distilled water. It was administered at a dose of 100mg/kg body weight orally for 10 days.

### Animals

Adult Swiss albino mice of either sex weighing 25-30 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The mice were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The mice were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water ad libitum. The mice were maintained on a 12:12 hour light-dark cycle.

### Assessment of Catalepsy

It was done at the Ethno pharmacology lab, Department of Pharmacology, Yenepoya University. Catalepsy was induced by I.P administration of haloperidol (1mg/kg). In order to measure cataleptic symptoms such as akinesia and rigidity, bar test was used. Catalepsy was evaluated by placing both forepaws of the mouse over a horizontal bar (diameter: 1 cm), elevated 4 cm from floor. The end point of catalepsy was considered to occur when the animal removed both the front paws from the horizontal bar (Vinod Nair et al, 2007).

### Experimental design

#### Effect of PAEE on Haloperidol induced catalepsy

Forty eight animals were used in this study. The animals were divided into four groups. Each group consisted of 6 males and 6 females (n=12).

- |           |  |
|-----------|--|
| Group I   | Distilled Water [DW] orally for 10 days  |
| Group II  | Distilled Water [DW] orally for 10 days + Haloperidol [H] I.P. on 10th day of the study.           |
| Group III | PAEE orally + Haloperidol I.P (1 hour after PAEE administration on 10th day).                      |
| Group IV  | Scopolamine I.P for 10days + Haloperidol I.P (1 hour after Scopolamine administration on 10th day) |

The test drug PAEE was administered orally for ten days. On tenth day, after one hour, haloperidol was given I.P to induce catalepsy. After half an hour of the administration of Haloperidol the animals were taken to measure the degree of catalepsy. Same pattern was followed for Scopolamine which was administered I.P. Once the experiments were over, the animals were rehabilitated.

### Statistical analysis

Results were expressed as mean  $\pm$  S.D. One-way analysis of variance (ANOVA) was carried out and the statistical comparisons among the groups were performed with Tukey Kramer multiple comparison test using Prism statistical package program.  $P < 0.05$  was considered significant.

## RESULTS

### Effect of PAEE on Haloperidol induced catalepsy

The data revealed that [table1], there was a considerable decrease ( $p < 0.001$ ) in cataleptic activity in PAEE and Scopolamine treated groups. It is also noteworthy that reduction in catalepsy state in plant extract treated group was highly significant ( $p < 0.001$ ) on comparing with the scopolamine treated group.

**Table 1: Effect of PAEE on Haloperidol induced catalepsy**

| Group            | Withdrawal of both front paws in minutes |
|------------------|--|
| I (Control)      | 0.49 $\pm$ 0.085                         |
| II (Haloperidol) | 35.2 $\pm$ 0.61 <sup>a</sup>             |
| III (PAEE)       | 0.52 $\pm$ 0.01 <sup>b,c</sup>           |
| IV (Scopolamine) | 2.7 $\pm$ 0.98 <sup>b</sup>              |

The Observations were analyzed by One Way ANOVA, followed by Tukey Kramer multiple comparison test, n = 12.  
a:  $p < 0.001$  extremely significant on comparing group II with group I  
b:  $p < 0.001$  extremely significant on comparing group III,IV with group II  
c:  $p < 0.001$  extremely significant on comparing III with group IV

## DISCUSSION

From the above results, it's clear that, indigenous medicinal plant, *Phyllanthus amarus* has the potential to prevent Parkinsonism. Haloperidol induced catalepsy is widely used as an experimental animal model of Parkinsonism (Manoj K. Aswar et al, 2010; Rasheed AS. et al., 2010). Catalepsy is a state which is characterized by idleness, reduced receptiveness to stimuli, and an inclination to sustain an immobile posture (Bhagya Manoj Sattigeri et al, 2009). Haloperidol induced catalepsy is due to the blockade of postsynaptic striatal dopamine D<sub>1</sub> and D<sub>2</sub> receptors (Manoj K. Aswar et al, 2010).

Parkinsonism, the neuro-degenerative disorder is associated with loss of dopaminergic neurons in substantia nigra pars compacta (Manoj K. Aswar et al, 2010). It's a well-known fact that the level of dopamine in the brain plays a major role in the etiology of this movement disorder (Reyhani-Rad S et al, 2012). The etiology of loss of dopaminergic neurons is not clear. The neurodegeneration of dopaminergic neurons associated with Parkinson's disease can be due to enormous oxidative stress, free radical formation and genetic vulnerability (Rasheed AS. et al., 2010). Apart from dopamine, the other neurotransmitter which plays a major role in the etiology of this progressive movement disorder is acetylcholine. Under usual conditions, acetylcholine release from the striatum is strongly repressed by dopamine. But in case of selective loss of dopaminergic neurons of substantia nigra, the inhibitory role of dopamine on acetylcholine release is stopped. This leads to the hyperactivity of cholinergic neurons and concomitant manifestations of Parkinson's disease (Galvan A et al, 2008). Reactive oxygen species (ROS) induced and free radical mediated oxidation of biomolecules have a role in a wide variety of pathological conditions including Alzheimer's disease and Parkinson's disease (Bhushan Gandhare et al, 2012). Recently the reports stating the involvement of other neurotransmitters i.e., serotonin and GABA in the etiology of Parkinson's disease has generated a great interest. Studies show that the serotonergic system plays a major role in the modulation of normal motor tasks. This effect is mediated through 5-HT<sub>1A</sub> receptors within the basal ganglia, which are located on dorsal raphe neurons with efferents to the striatum. 5-HT<sub>1A</sub> auto receptors exist on the serotonergic nerve endings in the raphe nuclei can lessen 5-HT synthesis and serotonergic transmission. Recently, a study showed that Buspirone, a partial agonist of 5-HT<sub>1A</sub> receptors, prevents catalepsy induced by 6-hydroxydopamine and haloperidol in animal models of Parkinson's disease. This effect is most likely caused by the increase in 5-HT<sub>1A</sub> receptor activation, resulting in an inhibition of serotonin release. Stimulation of 5-HT<sub>1A</sub> receptor is linked with an upsurge in dopamine turnover, dopaminergic cell firing and dopamine discharge. Thus, suggesting that 5-HT<sub>1A</sub> agonists have possible therapeutic importance in the management of Parkinson's disease (Reyhani-Rad S et al, 2012). Several animal models of Parkinsonism have revealed an altered level of GABA in brain. Muscimol a GABA agonist induced catalepsy.

This effect was dose-dependent. Muscimol catalepsy may be due to facilitating GABA inhibitory effect on dopaminergic function. Another animal study has shown that Gamma-hydroxybutyric acid, a metabolite of gamma-aminobutyric acid has potentiated the catalepsy induced by Tiapride, a selective D<sub>2</sub> antagonist. In another pre-clinical study on animals, Sodium valproate a broad spectrum anticonvulsant has potentiated the catalepsy induced by Haloperidol. This agent by the virtue of its GABA facilitating action might have stimulated the GABA receptors located on cell bodies of nigrostriatal dopaminergic neurons, thereby decreasing the release of dopamine into the synaptic cleft. Recently a magnetic resonance study has revealed that the level of GABA was increased in mild to moderate parkinsonian patients. These facts clearly signify the importance of GABA in the pathology of Parkinson's disease (Anne BY et al, 1984; Bhagya MS et al, 2009; Hikosaka O, 2007; Navarro et al, 1998; Sadeghj-LA et al, 1993; Schapira et al, 2006; Uzay EE et al, 2012). Alike other neurodegenerative disorders, in Parkinsonism too, free radicals play a central role in the pathogenesis. Degeneration in Parkinson's disease could be a result from the oxidative stress due to dysregulation of dopamine metabolism with subsequent free radical formation, diminution of reduced glutathione, increased level of total iron with reduced level of ferritin and scarcity of mitochondrial complex (Ravindra Pratap Singh et al, 2004; Nikolova G et al, 2012).

Catalepsy induced by haloperidol is due to the blockade of the dopaminergic receptors in substantia nigra. This in turn results in an unopposed action of acetylcholine in this region, which leads to cataleptic state (Vinod Nair et al, 2007). It is also a known fact that haloperidol induced catalepsy is due to oxidative stress arising from the generation of free radical catecholamine metabolism by monoamine oxidase (Parwez A et al, 2011)

Based on the above facts, the proposed mechanisms of actions for the anticataleptic effect of this plant are;

- Dopamine agonistic action
- Anticholinergic activity
- 5-HT<sub>1A</sub> agonistic activity
- GABA antagonistic activity
- Antioxidant activity.

## CONCLUSION

This preclinical study has proved that, *Phyllanthus amarus* has got a therapeutic role in Parkinsonism. The active constituent responsible for its anticataleptic action has to be identified. This will help in explaining the exact mechanism of its antiparkinsonian property.

**Conflict of Interest:** Nil

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