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Parkinson Disease And Its Pharmacological Evaluation By In-Vivo Methodes

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Abstract :-

Parkinson's Diseases (PD) , which is the second most common neurodegenerative disorder characterized by the progressive degeneration of the structure and function of the central nervous system. Parkinson Disease characterized by progressive death of dopaminergic neurons is substantia nigra. Parkinson's Diseases (PD) is commonly known to be a complex motor and non-motor multifocal neurodegenerative disorder. Parkinson's Diseases shows the various symptoms like rigidity ,tremor, bradykinesia. Parkinson's Diseases is the second most common illness, affecting 1% of those over the age of 55. Parkinson's Diseases shows the imbalance between Inhibitory Dopamine and Excitatory Acetylcholine.

INTRODUCTION

Parkinson's disease is the second most common progressive neurodegenerative disorder.The incidence of PD is more frequent in industrialized countries and was found to increase with aging. PD is common. It affects about 500,000- one million Americans, or about 1% of people over the age of 60. It typically develops between the ages of 55 to 65 years and above. Resulting from a pathophysiologic loss or degeneration of dopaminergic neurons in the substantia nigra of the midbrain and the development of neuronal Lewy Bodies. Other neurodegenerative disorders can mimic idiopathic PD. These include Dementia with Lewy Bodies (DLB), Corticobasal Degeneration (CBD), Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP).

Treatments of PD have focused until recently only the movements,but now we see the disorder in a more realistic, holistic way. When we talk about treating PD we currently only mean treating the symptoms. The actual disease is attack on the nerve cells in the brain, and to lesser extent outside the brain.

PD is associated with risk factors including aging, family history, pesticide exposure and environmental chemical use e.g. like synthetic heroin use. It is characterised by both both motor and non motor symptoms, PD patient classically display rest tremor, rigidity, bradykinesia and stooping posture.

Fig. Parkinson Disease person

SIGNS AND SYMPTOMS

There are two types of symptoms-

A. Motor
B. Non-motor

A. MOTOR SYMPTOMS

The motor symptoms of Parkinson's refer to those signs of the disease that affect the body's movement. There are four hallmark symptoms that are characteristic of Parkinson's, and are important for diagnosing the neurodegenerative disorder:

- Resting tremor
- Bradykinesia
- Rigidity
- Postural instability.

Resting tremor

The tremor consists of a shaking or oscillating movement, and usually appears when a person's muscles are at rest or relaxed, hence the term "resting tremor." Sometimes a hand tremor can be stopped by keeping the hand in motion or in a flexed grip. The tremor may get worse with stress or excitement and often spreads to the other side of the body as the disease progresses, but usually remains most apparent on the initially affected side.

In the early stages of the disease, about 70% of patients experience a slight tremor in the fingers, hand, or foot on one side of the body, or occasionally in the jaw or face.

Bradykinesia

Bradykinesia, or slowness of movement, is a defining feature of Parkinson's disease that involves a general reduction of spontaneous movement. This can give the appearance of abnormal stillness and a decrease in facial expressiveness.

Due to bradykinesia, patients with Parkinson's disease may have difficulties executing repetitive movements and performing everyday tasks, such as buttoning a shirt or brushing their teeth. People who experience bradykinesia may walk with short, shuffling steps. The reduction in movement caused by bradykinesia can affect a person's speech as the disease progresses

RIGIDITY

Stiffness or inflexibility of the muscles of the neck, shoulders, trunk, and limbs is common in Parkinson's and known as rigidity. It causes the affected muscles to remain stiff and not relaxed, decreasing their range of motion. Rigidity can be uncomfortable or even painful. A person with rigidity and bradykinesia cannot swing their arms while walking.

POSTURAL INSTABILITY

Postural instability, or impaired balance, is caused by the loss of reflexes that keep people in an upright position. Some patients develop a dangerous tendency to sway backward when rising from a chair, standing, or turning. This problem is called retropulsion and may result in a backward fall. Symptoms of postural instability usually develop later on in the course of the disease.

B. NON-MOTOR SYMPTOMS

The non-motor symptoms of Parkinson's include:

- Pain
- Fatigue
- Low blood pressure
- Restless legs
- Bladder and bowel problems
- Skin and sweating
- Sleep
- Eating, swallowing and saliva control
- Speech and communication issues
- Eye problems
- Foot care
- Dental health

Mental health issues

- Mild memory and thinking problems
- Anxiety
- Dementia
- Depression

- Hallucinations and delusions

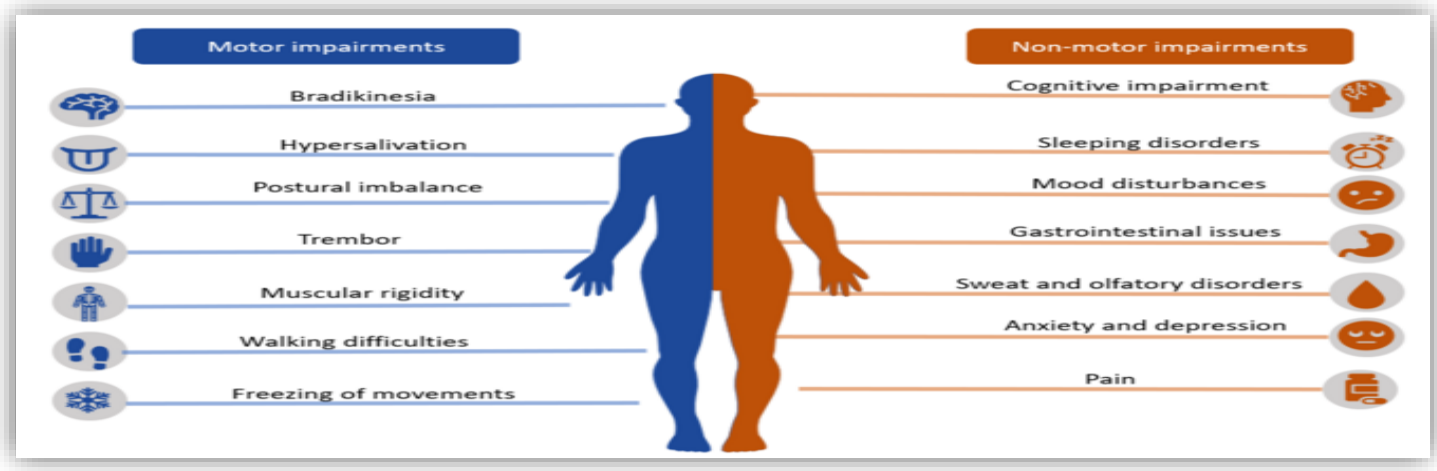


Fig.no. Motor and Non-motor symptoms

TREATMENT

Although there is no cure for Parkinson's disease, medicines, surgical treatment, and other therapies can often relieve some symptoms.

These treatment includes:

1. Medication
2. Surgery
3. Life style and home remedies

1. MEDICATION

Medication can be used to improve the main symptoms of Parkinson's Disease, such as shaking and movement problems.

<u>Antiparkinson Drugs:</u>

<u>Drugs affecting brain dopaminergic system-</u>

Dopamine precursor- Levodopa

Peripheral decarboxylase inhibitor- Carbidopa

COMT inhibitors- Entacapone

MAO-B- Selegiline

Dopaminergic agonist- Bromocriptine

Drugs affecting brain cholinergic system-

Central anticholinergics- Procyclidine

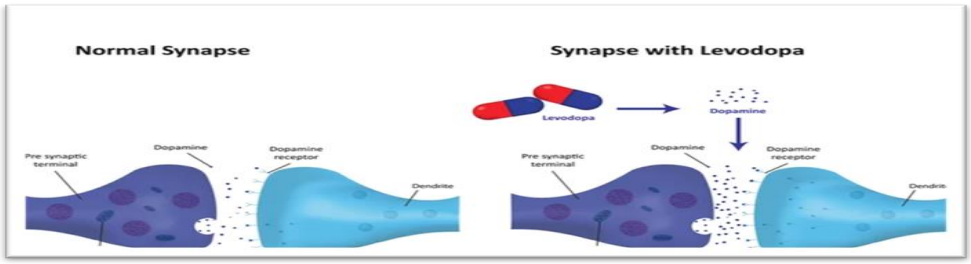
Antihistaminics- Promethazine

Levodopa (Dopamine precursor)

Levodopa is the precursor to dopamine. Most commonly, clinicians use levodopa as a dopamine replacement agent for the treatment of Parkinson disease. It is most effectively used to control bradykinetic symptoms that are apparent in Parkinson disease, and it is the most effective medication to improve the quality of life in patients with idiopathic Parkinson disease. Levodopa is typically prescribed to a patient with Parkinson disease once symptoms become more difficult to control with other antiparkinsonian drugs.

Mechanism of action

Degeneration of the substantia nigra occurs in patients with Parkinson disease. This condition results in the disruption of the nigrostriatal pathway and thus decreases the striatal dopamine levels. Unlike dopamine, levodopa can cross the blood-brain barrier (BBB). Levodopa converts to dopamine in both the CNS and periphery. To increase the bioavailability of levodopa and decrease its side effects, it is often administered in combination with peripheral decarboxylase inhibitors (such as carbidopa). Dopamine decarboxylase inhibitors prevent the conversion of levodopa to dopamine in the periphery, allowing for more levodopa to cross the BBB. Once converted to dopamine, it activates postsynaptic dopaminergic receptors and compensates for the decrease in endogenous dopamine.



PHARMACOLOICAL EVALUATION FOR IN-VIVO METHODS

In Vivo Methods :

- **ICV Method**

Animals will be anesthetized with sodium thiopental (60 mg/kg, 10 ml/kg, i.p). In a stereotaxic apparatus, the skin of the skull will be removed, and an i.c.v. guide cannula for infusion will be implanted. Stereotaxic coordinates were 1.5 mm posterior to bregma, 1 mm right of the midline. The guide cannula will be implanted 1.7 mm ventral to the superior surface of the skull and fixed with jeweler's acrylic cement. Experiments will be performed 48 h after surgery. i.c.v. treatments will be performed with a 30-gauge

cannula, which will be fitted into the guide cannula and connected by a polyethylene tube to a micro syringe. The tip of the infusion cannula protruded 1 mm.

a. Sodium Nitrite Induced Animal Model

Sodium Nitrite induce PD model was tried but no significant changes were observed in the selected animal models. Hence, this model was not considerd further for the study. (result not shown).

b. Aluminium Chloride Induced Animal Model

Animals were divided into respective groups in each group having six animals All experimental procedures were carried out under strict compliance with Institutional Animals Ethical Committee.

c. Grouping :-

Standardization Aluminium Chloride Induced Model

- Standardization of Aluminium induced Parkinson like condition was done by intraperitoneal injection of $AlCl_3$ (20 mg/ kg) for 10 days but no significant induction was observed (results not shown)
- Hence the induction was tried by ICV administration of $AlCl_3$ (5 µg / kg).

Group I :- Control group (Received only 0.9 % Saline Solution).

Group II :- Induction group (Aluminium Chloride 5 µg / kg ICV for 5 Days).

- The effect of curcumin and piperine and their combination was evaluated by simultaneous administrat of test drugs as per the prevention protocol.

Effect of Curcumin and Piperine on Aluminium Induced Model :-

Group I :- Control group (Received only 0.9 % Saline Solution)

Group II :- Induction group (Aluminium Chloride 5 µg / kg ICV for 5 Days).

Group III :- Standard group (Levodopa and Carbidopa Tablets 100 mg/kg).

Group IV and V :- Curcumin solution was freshly prepared in DMSO.(Dose 25mg /kg, 50mg/kg).

Group VI and VII :- Piperine solution was freshly prepared in DMSO. (Dose 10mg/kg, 20mg/kg).

Group VlII :- Combination Group (Curcumin 25 mg/kg + Piperine 10 mg/kg)

d. Locomotor activity by Actophotometer: -

The locomotor activity can be easily measured using an actophotometer. which operates on photoelectric cells which are connected in circuit with a counter. when beam of light falling on the photocell is cut off by the animal, a count is recorded. The actophotometer experimentally weight of the animal and number them and turn on equipment and place individually each mouse in the activity cage for 10 min and the difference in the activity, before and after drug. **(Kulkarni, 2005).**

Fig. No. 11 Actophotometer

e. Evaluation of paw Strength & Grip by Rotarod: -

For Parkinson disease models, a full assessment of the motor deficits must include specific tests of strength. Place the mouse in the center of the wire mesh screen, start a stopclock, rotate the screen to an inverted position over 2 sec, with the rats head declining first. Hold the screen steadily 40-50 cm above a padded surface. Note the time when the rats falls off, or remove it when the cutoff time of 60 sec is reached. Longer criterion times may be useful for some experiments. 3. Scoring the inverted screen: Falling between 1-10 sec = 1 Falling between 11-25 sec = 2 Falling between 26-60 sec = 3 Falling after 60 sec = 4 Or, e.g. for 2 min: Falling between 1-10 sec = 1 Falling between 11-25 sec = 2 Falling between 26-60 sec = 3 Falling between 61-90 sec = 4 Falling after 90 sec = 5. **(Kulkarni, 2005).**

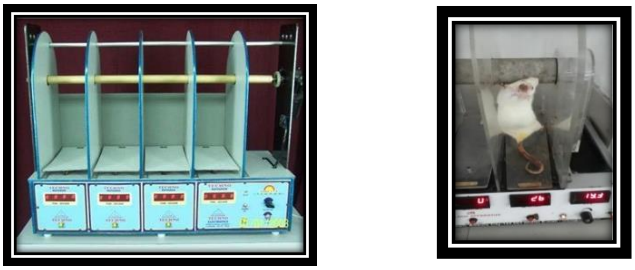


Fig. No. 12 Rotarod

f. Rigidity by Catalepsy model:-

The primary motor symptoms of patients with PD are muscular rigidity and akinesia-bradykinesia and resting tremor **(Paul et al., 1988).** The time for which the rats remains on the bar was calculated. Cut of time was recorded.

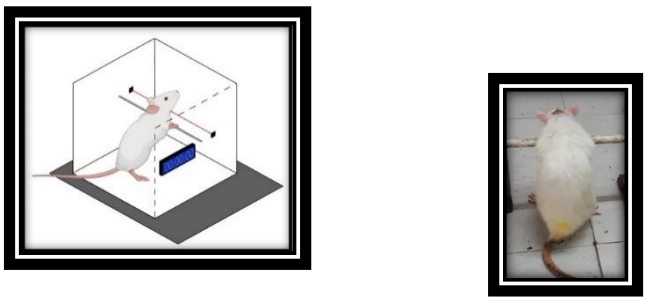


Fig. No. 13 Rigidity by Catalepsy

g. Gait by Paw Print Method :-

To obtain the pawprints, the rat hindlimb and forelimb were coated with green and red non-toxic paints. The animals were then allowed to walk along a 100 cm long, 10cm wide runaway (with 20 cm high walls). A fresh sheet of white paper was placed on the floor of the runaway for each rat run. The pawprint patterns were analyzed for three step parameters (all measured in centimeters): stride length, base width and overlap between forelimb and hind limb. A sequence of four consecutive steps was chosen for evaluation, pawprints made at the start and end of the run where the animal was initiating and finishing movements. **(Syeda et al., 2017).**

Fig. No. 14 Paw print method

RESULTS :- Statistical Analysis :-

Graph Pad Prism Software (Version 9.1.2) was used to analyse all quantitative data. The data was analysed using Two-way ANOVA followed by Bonferroni's Post-Hoc Test, and the results were reported as Mean SEM. P values were calculated for the statistical significance between the treatment and induce groups (*P 0.05, **P 0.01, ***P 0.001).

- **Standardisation Result**

Values shows (Mean ± SEM)

Days	Control	$AlCl_3$ Induce Group
1	329.5 ± 2.38	129.5 ± 19.36
2	321.25±20.39	101±8.32
3	323.5±5.74	89.25±9.10
4	329.25±4.85	63.5±8.38
5	331±0.81	53±3.16***

Table No. 7 Actophotometer

Days	Control	$AlCl_3$ Induce Group
1	89.7±0.94	59.5±4.19
2	90. 5±0.95	46.5±4.04
3	89±0.84	38.25±2.5
4	89.5±0.57	21.25±6.16
5	88.5±0.57	13.5±2.38***

Table No. 8 Rotarod

Days	Control	$AlCl_3$ Induce Group
1	2.5± 0.5773	26.5±3.10
2	2.75±0.9574	30.5±0.53
3	2.75±0.5	51.75±3.59
4	2.75±o.95	87.5±2.08
5	3.5±0.57	92.5±2.38***

Table No. 9 Rigidity by Catalepsy

Days	Control	$AlCl_3$ Induce Group
1	6.125 ± 0.05	5.25 ±0.0
2	6.15± 0.05	4.525 ±0.49
3	6.15± 0.07	4.175 ±0.51
4	6.15± 0.10	3.525 ±0.28
5	6.15±0.05	2.275 ±0.46***

Table No.10 Paw Print Method

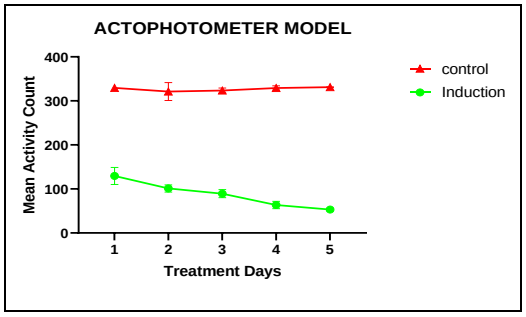


Fig. No. 15 Standardization of Alcl3

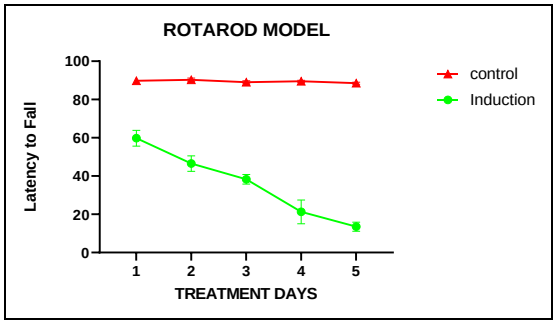


Fig. No. 16 Standardization of Alcl3

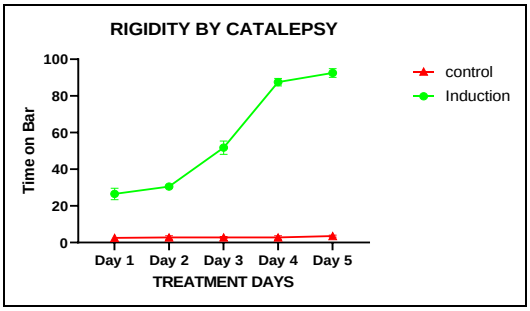


Fig. No. 17 Standardization of Alcl3

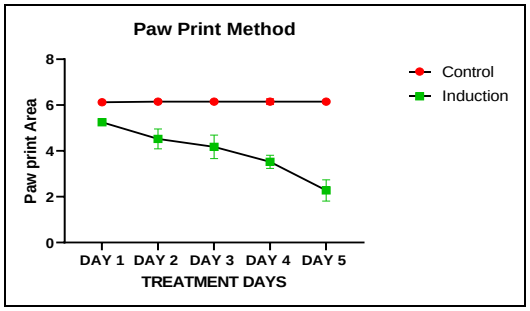


Fig. No. 18 Standardization of Alcl3

Values are expressed in Mean ± SEM. Control group compared with induction group. The values are analyzed in Graph-pad Prism followed by Two-way ANOVA and ***: P ≤0.001was considered to be statistically significant.

Effect of treatment on actophotometer count in Aluminium chloride induce Parkinson diseases models

Day's	Control (Count)	Induction (Count)	Standard (Count)	Treatment (Curcumin 25 mg)	Treatment (Curcumin 50 mg)	Treatment (Piperine 10 mg)	Treatment (Piperine 20 mg)	Combination
1	329.5±2.38**	122±13.78***	231±10.89	139.5±4.65	151±15.76	172.75±7.80	176.25±10.0	229±0.81**
2	321.25±20.3	99.75±6.5	180.2±2.21	114.25±4.5	118.75±5.5	132.75±9.10	141.5±11.90	247.5±1.29
3	323.5±5.74	83±5.163	145.25±22.9	93.5±4.43	99.5±6.55	101.25±7.93	105.25±4.42	266±2.58
4	329.25±4.85	61±5.163	101.7±3.09	80.25±4.57	83.75±3.86	77.25±8.09	90±6.271	287.75±1.25
5	331±0.81**	54±4.396**	87.5±8.88	60.75±7.63	73.5±4.43	61±4.32	73±3.651	296.25±1.25***

Table No. 11 Actophotometer

Values shows Mean Activity Count (Mean ± SEM). * Induction group is compared with control group and treatment group are compared with induction groups. # 1st day readings are compared with 5th day reading. *P< 0.05; **P< 0.01; ***P<0.001; ****P<0.0001. Statistical analysis was done using Two- Way ANOVA by graph pad prism (Version 9.1.2). ****P<0.0001 was considered statistical significant.

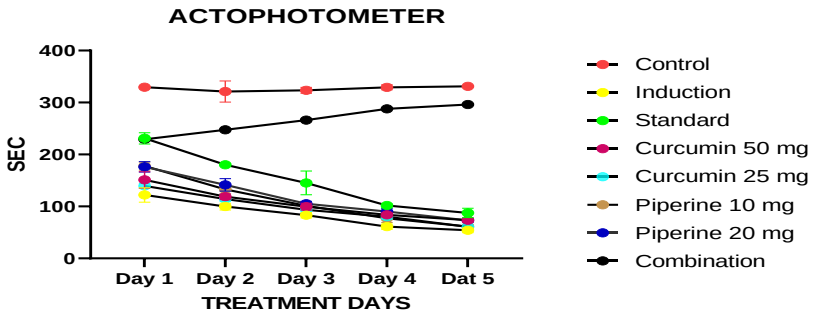


Fig. No. 19 Actophotometer

The locomotion count was decreased in induction group as compared to control on 1st day indicating rigidity. The count of induction group on 5th day was significantly less as compared to 1st day. This shows that AlCl3 induces rigidity in 5 days. In the treatment groups with higher dose, the count was decreased but on 5th day as compared to day 1. This means Curcumin and Piperine alone was effective in reducing rigidity and in combination the count was significantly increased on 5th day than 1st day indicating reduced rigidity. The combination effect was highly signicant.

Effect of treatment on rotarod latency in Aluminium chloride induce Parkinson diseases models

Day's	Control (fall of time)	Induction (fall of time)	Standard (fall of time)	Treatment (Curcumin 25 mg)	Treatment (Curcumin 50 mg)	Treatment (Piperine 10 mg)	Treatment (Piperine 20 mg)	Combination
1	89.75±0.95**	55.2±4.53***	81.5±10.14	17.5±0.57	19±1.41	17.25±0.95	18.25±2.25	58±1.356**
2	90.25±0.95	45.5±3.42	54.5±5.90	13.5±0.57	15±0.81	11.5±0.57	13.25±0.94	106±2.47
3	89±0.81	33.5±3.10	39.5±10.47	11.25±0.95	12.25±1.70	8.5±0.57	9.75±1.5	110±1.15
4	89.5±0.57	20.5±4.79	19±3.55	7.5±0.57	9±1.13	6±0.81	6.25±0.95	128.5±3.3
5	88.5±0.56**	12.7±2.75**	10.5±1.29	5.25±0.95	5.75±0.950	2±0.0	3.25±0.5	139±4.00***

Table No. 12 Rotarod

Values shows Fall off Time in Sec (Mean ± SEM).* Induction group is compared with control group and treatment group are compared with induction groups. # 1st day readings are compared with 5th day reading. *P< 0.05; **P< 0.01; ***P<0.001; ****P<0.0001. Statistical analysis was done using Two- Way ANOVA by graph pad prism (Version 9.1.2). ****P<0.0001 was considered statistical significant.

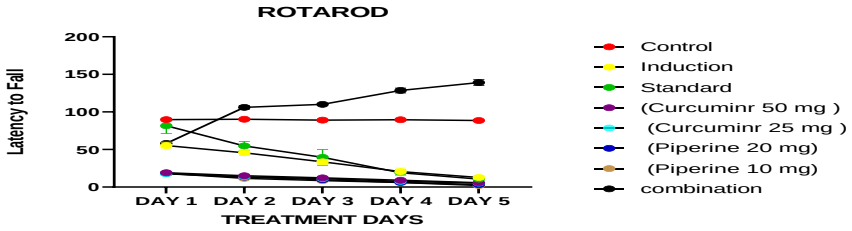


Fig. No. 20 Rotarod

The fall off time was decreased in induction group as compared to control on 1st day indicating rigidity. The fall off time of induction group on 5th day was significantly less as compared to 1st day. This shows that $AlCl_3$ induces rigidity in 5 days. In the treatment groups with higher dose, the fall off time was decreased on 5th day as compared to day 1. This means Curcumin and Piperine alone was effective in reducing rigidity but with combination, increase in the fall off time was highly significantl on 5th day than 1st day indicating decreased rigidity.

- **Effect of treatment on rigidity by catalepsy in Aluminium chloride induce parkinson diseases models**

Day's	Control (Time on Bar)	Induction (Time on Bar)	Standard (Time on Bar)	Treatment (Curcumin 25 mg	Treatment (Curcumin 50 mg)	Treatment (Piperine 10 mg)	Treatment (Piperine 20 mg)	Combination
1	2.5±0.57**	16.75±0.957****	13.25±0.957	16.5±2.0	18.5±1	11.75±0.95	14.75±2.06	21.75±1.5***
2	2.75±0.92	18.25±0.957	46.75±2.872	37.5±3.41	39.75±o.957	27.5±1.29	31±1.413	17.25±0.957
3	2.75±0.5	49±0.965	53.5±1.732	50±2.16	60±1.825	60±1.82	70±1.415	14.25±0.957
4	2.75±0.92	87.5±2.059	55.25±2.98	61.75±2.75	68±1.821	67.5±1.91	77.25±1.25	11.5±1.294
5	3.5±0.50**	90.25±0.957***	60±1.154	70.25±2.21	71.25±0.5	76.75±2.21	78.25±0.95	10.25±0.910**

Table No. 13 Rigidity by Catalepsy

Values shows Time On Bar (Mean ± SEM). * Induction group is compared with control group and treatment group are compared with induction groups. # 1st day readings are compared with 5th day reading. *P< 0.05; **P< 0.01; ***P<0.001; ****P<0.0001. Statistical analysis was done using Two- Way ANOVA by graph pad prism (Version 9.1.2). ****P<0.0001 was considered statistical significant.

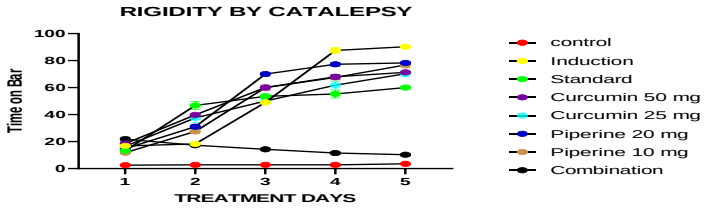


Fig. No. 21 Rigidity by catalepsy

The time on bar was increased in induction group as compared to control on 1st day indicating rigidity. The increase in time on bar of induction group on 5th day was significant as compared to 1st day. This shows that $AlCl_3$ induces rigidity in 5 days. In the treatment groups with higher dose, the time on bar was decreased less significantly on 5th day. This means Curcumin and Piperine alone was effective in reducing

rigidity but in combination the time was decreased with high statistical significance on 5th day than 1st day indicating decreased rigidity.

- **Effect of treatment on paw print area in Aluminium chloride induce parkinson diseases models**

Day's	Control (Paw print area in cm)	Induction (Paw print area in cm)	Standard (Paw print area in cm)	Treatment (Curcumin 25 mg)	Treatment (Curcumin 50 mg)	Treatment (Piperine 10 mg)	Treatment (Piperine 20 mg)	Combination
1	6.125±0.05**	5.25±0.057**	5.45±0.364	3.85±0.09	4.1±0.51	3.85±0.65	3.85±0.654	6.9±0.081**
2	6.15±0.057	4.525±0.43	4.625±0.499	3±0.11	3.375±0.37	3.675±0.69	3.675±0.69	7.275±0.125
3	6.15±0.055	4.175±0.51	3.975±0.679	2.45±0.17	2.525±0.250	3.55±0.45	3.55±0.497	7.925±0.049
4	6.15±0.194	3.525±0.28**	4.075±0.66	2.075±0.26	2.4±0.145	2.275±0.17	2.275±0.12	7.975±0.498
5	6.15±0.055**	2.275±0.464	3.475±0.275	1.325±0.15	1.65±0.261	1.8±0.08	1.8±0.086	8.3±0.141***

Table No. 14 Paw Print Method

Values shows distance between the Paw Print Area in cm (Mean ± SEM).* Induction group is compared with control group and treatment group are compared with induction groups. # 1st day readings are compared with 5th day reading. *P< 0.05; **P< 0.01; ***P<0.001; ****P<0.0001. Statistical analysis was done using Two- Way ANOVA by graph pad prism (Version 9.1.2). ****P<0.0001 was considered statistical significant.

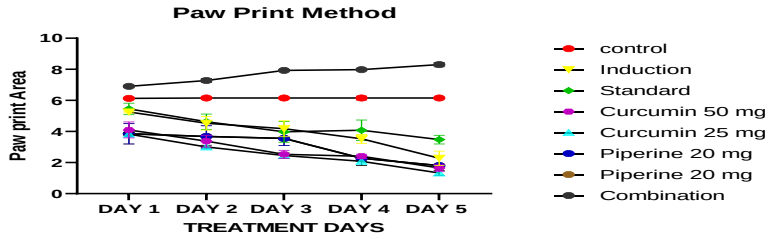


Fig. No. 22 Paw print method

The distance between the paws was decreased in induction group as compare to control on 1st day indicating rigidity. The distance between the paws induction group on 5th day was significantly less as compared to 1st day. This shows that $AlCl_3$ induces PD like gait in 5 days. In the treatment groups

with high doses, the distance between the paws was increased on 5th day. This means Curcumin and Piperine alone was effective in reducing gait changes but in combination the gait were more significantly normalized as indicated by change in paw distance on 5th day.

7. DISCUSSION

In this work, we looked at the behavioural and neuropathological effects of exposing rats to Aluminium Chloride and sodium nitrite in the lab. Sodium nitrite induce PD model was not successful as the induction group did not show significant effects in the behavioural models. We found that the $AlCl_3$ induced animals had changed neurological parameters, as compared to control animals. Standardization of the Aluminium-induced Parkinson-like condition was initially tried by injecting $AlCl_3$ (20 mg/ kg) by intraperitoneal route for 10 days, but no significant induction was detected (results not shown). Hence, the induction was attempted using ICV administration of $AlCl_3$ (5 µg/ kg) for 5 days which showed movement abnormalities, behavioural alterations, and histological changes in the brain that are identical to those seen in Parkinson's disease.

Aluminium chloride is a neurotoxic that plays a role in the growth of a variety of mental illnesses. Aluminium chloride causes long-term movement impairment due to rigidity and oxidative stress. In this research, we used Actophotometer, Rotarod, Catalepsy Model for rigidity, and Paw Print Method to measure various characteristic parameters indicative of PD like condition (Tsuchiya et al.,2013). Locomotion can be affected in PD like condition due to rigidity and hence in actophotometer the locomotion count is reduced in the induction group which may be reversed by antiparkinson drug. In rotarod, the fall off time of animal is reduced in rigid animals as the animal is not able to hold the bar due to muscle rigidity and tremors. In catalepsy bar model, the time for which the animal remains on bar is increased due to increased rigidity and it may be reduced by antiparkinsonian drug. The gait of the PD patient is altered which shows smaller steps than normal. Animal gait can be studied by paw print method. The distance between the two paws is less in induction group and the gait becomes normal with normal paws distance in case of treated animals. Aluminium chloride reduced the actophotometer count, increased rotarod grip strength, increased stiffness and rigidity in catalepsy model and decreased paw distance as compared to control group indicating the PD like symptoms. The treatment with curcumin and piperine alone reversed these observations less significantly. But the combination of curcumin and piperine showed more significant action on the above parameters (Khemani et al.,2014).

CONCLUSION

Hence it can be a promising curative agent to treat the PD in the future. However, the further research work was still needed in the future to understand the exact mechanism of curcumin and piperine against the PD.

Future Scope

- ❖ The in vitro antioxidant and anti-inflammatory assays for combination of curcumin and piperine can be additionally performed.
- ❖ The biochemical estimations for oxidative stress enzymes and some neurotransmitters can be further performed to support the in-vivo behavioral, in vitro anti-inflammatory activity and histopathological studies.
- ❖ The further research work can be done in future to understand the exact mechanism of curcumin and piperine against the PD.
- ❖ The similar studies can be performed with other phytonutrients.

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