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Comparative effect of *Centella asiatica* and levodopa on haloperidol induced Parkinsonism in W*istar albino* rats

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Abstract: **The present study was to evaluate the effectiveness of aqueous preparations of *Centella asiatica* in the management of haloperidol induced Parkinsonism in rats. Haloperidol is an antipsychotic medication and functions as an antagonist of dopamine. As a result, the animal is induced with Parkinsonism. It was administered to the rats as intraperitoneal injection at a dose of 1 mg/kg body weight, 4 hours and 30 minutes prior to the start of the behavioural experiment. A total of 36 *Wistar albino* rats were screened for this study. All the test animals weighed between 180 - 200 grams. Animals were grouped into General, Control and Test. General group were normal animals. Control group 1 consisted of haloperidol injected (intra peritoneally 1 mg/kg body weight) and Control group 2 consisted of *Centella asiatica* fed orally (100 mg/kg body weight). The test group 1 was treated with haloperidol + *Centella asiatica* and test group 2 with haloperidol + levodopa (10 mg/kg body weight). Progression of the disease was detected with rotarod, beam walking, open field and foot print analysis. L-dopa is used in the clinical treatment of Parkinson's disease and dopamine responsive dystonia. It was fed to the rats by orogastric feeding tube at a dosage of 10 mg/kg body weight, 6 hours and 30 minutes prior to the start of motor analysis. The values were statistically analyzed for significance using Turkey post-hoc test (SPSS verson 16). Results revealed that aqueous preparation of *Centella asiatica* had a significant role in the management of Parkinson's disease. There is a significant difference in between the control and the test groups. Within the test groups, test group 2 (Levodopa treated animals) performed better than the test group 1 (animals fed with *Centella asiatica*).**

Keywords: **Parkinson's disease, *Centella asiatica*, Levodopa, haloperidol, dopamine.**

1. INTRODUCTION

Parkisonism: Parkinson's disease is a chronic, progressive and age-dependent neurodegenerative movement disorder. It is characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta region of the midbrain that culminates in the major clinical symptoms of PD. Dr.James Parkinson in a little book entitled " Essay on the shaking palsy " published in 1817 first described the clinical syndrome that was later to bear his name [1]. The etiology of the neuronal cell death is still unclear, but genetic mutations, abnormal handling of misfolded proteins by the ubiquitin-proteosome, autophagy – lysosomal systems, increased oxidative stress, mitochondrial dysfunction, inflammation and other pathogenic mechanisms have been identified as contributing factors in the death of dopaminergic and non-dopaminergic cells in the brains of patients with PD [2]. The disease is characterised by bradykinesia, rigidity, postural instability, orofacial dyskinesia, muscular stiffness and tremor. Other non motor complications include sleep disorders and cognitive impairment [3], depression [4], mood fluctuations [5], psychosis and dementia [6], poor sense of smell, constipation, fatigue and other impairments that also accompany Parkinson's disease.

Levodopa: L-DOPA (1,3,4- dihydroxy phenyl alanine) is a chemical made and used as part of medical treatment. It is the first line of treatment for Parkinsonism. It is made through the biosynthesis from amino acid L-Tyrosine. L-DOPA is the precursor of neurotransmitters dopamine, norepinephrine, ephinephrine collectively known as catecholamines. Levodopa is the most effective drug used in the treatment of Parkinsonism and dopamine responsive dystonia.

Haloperidol: Haloperidol or haldol is a psychotropic drug of the butyrophenone family. It functions as an inverse agonist of dopamine. It is discovered by Paul Janssen. It is taken orally or intravenously. It is used for both chronic and short term therapy. Long-term therapy is commonly used for psychotic disorders such as schizophrenia, senile psychosis or the manic phase of bipolar disorders. Short -term therapy is used in acutely confused states including the relief of delusions, delirium and aggressive behavior. Although haloperidol appears to function by blocking dopaminergic neurotransmission in the central nervous system, the precise mechanism for its therapeutic effects remains unknown. Half-life of haloperidol is 17 to 18 hours [7] and it may exert prolonged effects [8]. Haloperidol is easy to use and effective in controlling acute delirium and combative states. Overdose leads to dystonia, muscle rigidity, akathesia, Parkinsonism, hypotension, dry mouth, constipation and blurred vision. It may also cause permanent movement disorders. It affects the foetus when taken during pregnancy.

Centella asiatica: *Centella asiatica* commonly known in Malayalam as *Gotu kola, Kodangal, Karinga, Muttil* etc. It is a native tropical plant, also called Asiatic pennywort, Indian water navelwort, wild violet and tiger herb. The plant is indigenous to South east Asia, India, Srilanka, China, Madagascar, South Africa, South America etc, which has also cultivated successfully due to its medical importance in some countries including Turkey and traditionally, it has been used as a brain tonic in Ayurvedic and Chinese medicines [9]. *C. asiatica* has been reported to have a comprehensive neuroprotection by different modes of action such as enzyme inhibition, prevention of amyloid plaque formation in Alzheimer's disease, dopamine neurotoxicity in Parkinson's disease and decreasing oxidative stress. Therefore, *C. asiatica* could be suggested to be a desired phytopharmaceutical with neuroprotective effect emerged from traditional medicine.

2. MATERIALS AND METHODS

1. EXPERIMENTAL ANIMALS: A total of 36 Wistar albino rats were screened for the study. Body weights, eating habits, walking patterns of the test animals were analyzed. All the test animals weighed 180 - 200 grams. They were maintained in the animal house of the Department of Lifesciences (Reg # 426/2/CPCSEA) and the Institutional Animal Ethical Committee clearance was obtained. Rats were housed in polypropylene cages of dimension 29 x 22 x 14 cm with wire mesh lids. Paper strips were used as bedding material. Food and water were provided *ad libitum*.

2. HALOPERIDOL: HALDOL (haloperidol) is available as a sterile parenteral form for intra peritoneal injection. The injection provides 5 mg haloperidol (as lactate) and lactic acid for pH adjustment between 3.0 - 3.6. The adverse effects of haloperidol are Parkinsonism, hypotension, constipation, dry mouth and blurred vision. It is an antipsychotic medication and function as an antagonist of dopamine. It was administered to the rats as intraperitoneal injection at a dose of 1 mg/kg body weight, 4 hours and 30 minutes prior to the start of behavioural experiments.

3. COLLECTION OF PLANT LEAVES FOR EXTRACTION: The fresh plant of *Centella asiatica* was collected from Malappuram district, Kerala during the month of September, 2016. The taxonomic identify of the plant was confirmed by comparing collected specimen with those of known identity which are located in the Herbarium (Acc. No. 6893) of the Department of Botany, University of Calicut, Kerala. The leaves were separated from the plant and washed thoroughly with water before use.

4. PLANT EXTRACT PREPARATION*:* The leaves of *Centella asiatica* were used to prepare the extract. Fresh extract was made by grinding the leaves using a mortar and pestle (100 mg leaves in 10 ml distilled water). It was fed to the rats by orogastric feeding tube at a dosage of 100 mg/kg body weight, 6 hours and 30 minutes prior to the start of motor analysis.

5. LEVODOPA: L-DOPA [L 3, 4- dihydroxyphenyl alanine] is a chemical that is made and used as part of medical treatment. L-DOPA is the precursor to the neurotransmitter dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) collectively known as catechol amines. It is used in the clinical treatments of Parkinson's disease and dopamine responsive dystonia. It was fed to the rats by orogastric feeding tube at a dosage of 10 mg/kg body weight, 6 hours and 30 minutes prior to the start of motor analysis.

6. ROTAROD: A rotating drum of 8 cm diameter was suitably roughened to allow the rats to grip on it. Rats were placed on the rod while it was turned at a constant speed. When the rotarod was rotated, the rats have to walk continuously. The maximum time alloted for walking along the rotarod was fixed as 180 seconds. The length of time that the rats stay on this rotarod is a measure of their balance, coordination, physical condition, motor activity etc. The speed of the rotarod is motor driven, which may be either held constant or accelerated. Test evaluates balance and sensory motor coordination of the subjects.

7. BEAM WALKING A narrow beam was constructed for analyzing the movement pattern of rats. It has a dimension of 100 x 3.5 cm and is placed 75 cm above the floor with the help of two poles. The rats were allowed to walk along the beam and the time taken for reaching the mark, which is previously noted in the beam is recorded. The animals are first pretrained. This helps to make sure that the behavior during the test is more stable and more accurately reflects motor coordination as opposed to the rodent 's natural aversion to crossing over unprotected spaces. After pretraining the animals, it can be tested on the balance beam for the latency to cross the beam and also the number of legs.

8. FOOT PRINT ANALYSIS: This test is based on analysis of foot prints, which is a very good indicator of walking stablility and body balance by analyzing Sciatic Function Index (SFI). The foot print of rats were analyzed by using a walking track ending in a darkened cage. The dimension of corridor of walking track was 45 x 12.5 cm. The same dimensioned paper strip was placed on the corridor of the walking track. Fabric paint was applied on the hind limbs of the rats for getting foot prints. This corridor was set up in the laboratory by using card board and paper. The rats were permitted to walk down the track leaving its hind foot prints on the paper. Immediately after taking the foot print the feet of the rats were cleaned with water. The paper was air dried. From the foot print, the following parameters were obtained, which is used for analyzing the sciatic nerve function in rats known as Sciatic Function Index.

Distance from heel to the top of the third toe (Print Length = PL). Distance between the first and fifth toe (Toe Spread = TS). Distance from the second to the fourth toe (Intermediary Toe Spread = ITS). All the three measurements were taken from the Experimental (E) and Normal (N) groups. The factors were calculated as follows,

Print length factor (PLF) = (EPL – NPL) / NPL

Toe spread factor (TSF) = (ETS – NTS) / NTS

Intermediary toe spread factor (ITF) = (EIT- NIT) / NIT

9. OPEN FIELD ANALYSIS

This test provides simultaneous measures of locomotion, exploration and anxiety. The lines divide the floor into twenty five 18 x 18 cm squares. The experimental animal was placed at the center of the open field and allowed to explore the apparatus for 5 minutes. After this, the rats were returned to their home cages and the open field was cleaned with ethyl alcohol and permitted to dry between the tests. The following parameters were recorded to analyze the motor activities.

- Line crossing : Frequency with which the rats crossed the lines with all the four paws.
- Rearing : Frequency with which rats stood on their hind legs in the maze
- Each animal was scored for their total locomotor activity that was calculated as to some of line crosses and number of rearings.

10. OTHER LABORATORY INSTRUMENTS

Routine laboratory instruments like syringes, scissors, cotton, pipette, mortar and pestle etc., were also used.

3. METHODS

A total of 36 Wistar albino rats were screened for the study, which have an average body weight of 180 - 200 grams, they were grouped into general, control and test groups. General group considered to be normal treated with normal diet, control 1 group was treated with Haloperidol (Disease induced), control 2 group was treated with *Centella asiatica*, control 3 group was treated with *Levadopa alone*, test group 1 was treated with Haloperidol + *Centella asiatica* and test group 2 was treated with Haloperidol + *Levadopa alone.*

The following experiments were carried out after the above mentioned treatments in all the groups

1. Rotarod 2. Beam walking 3. Footprint analysis 4. Open field analysis

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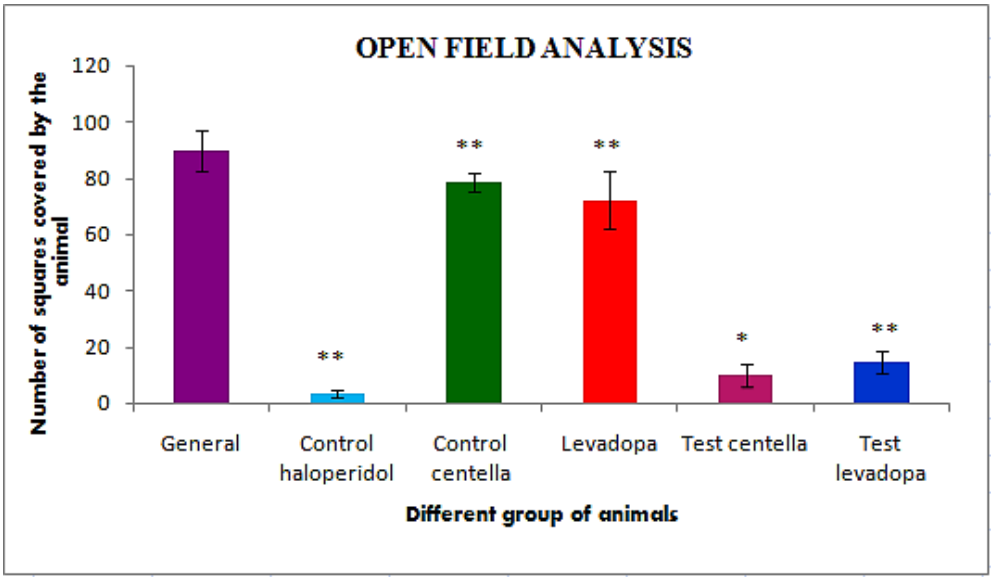
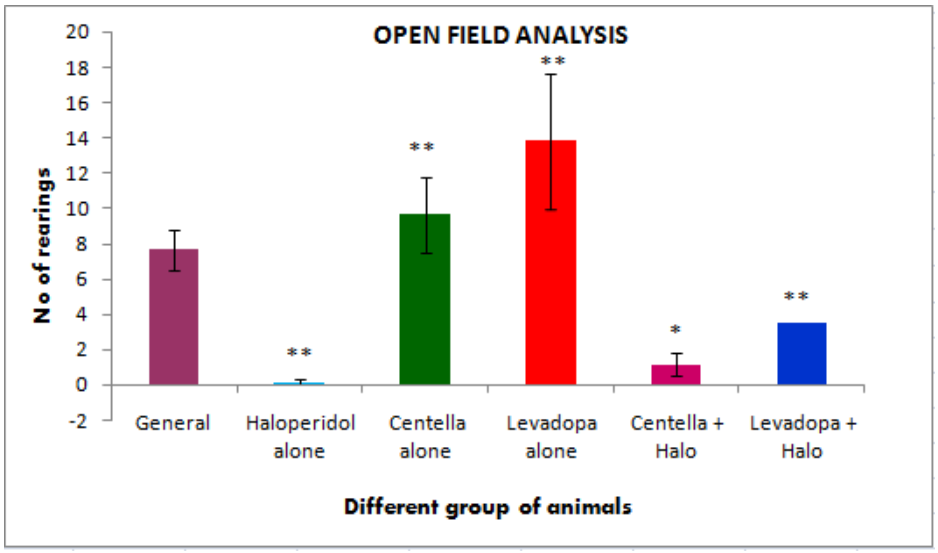
4. RESULTS



Graph 1: Open field exploratory scores of different group of animals (n=6).

****Significant at 1% level. ***Significant at 5% level.

The above graph shows the comparisons of the open field exploratory scores of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed a decline in the scorings than the other groups. The test group 1 (haloperidol + *Centella asiatica*) showed a decreased level of scoring when compared with the general group. Test group 2 (haloperidol + levodopa) is significantly better than the test group 1 (haloperidol + *Centella asiatica*).



Graph 2: Open field rearing score of different groups of animals (n=6).

****Significant at 1% level. ***Significant at 5% level.

The above graph shows the comparisons of the open field rearing scores of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed a decline in the scores when compared with the general group. Test group 2 (haloperidol + levodopa) is significantly better than the test group 1 (haloperidol + *Centella asiatica*).

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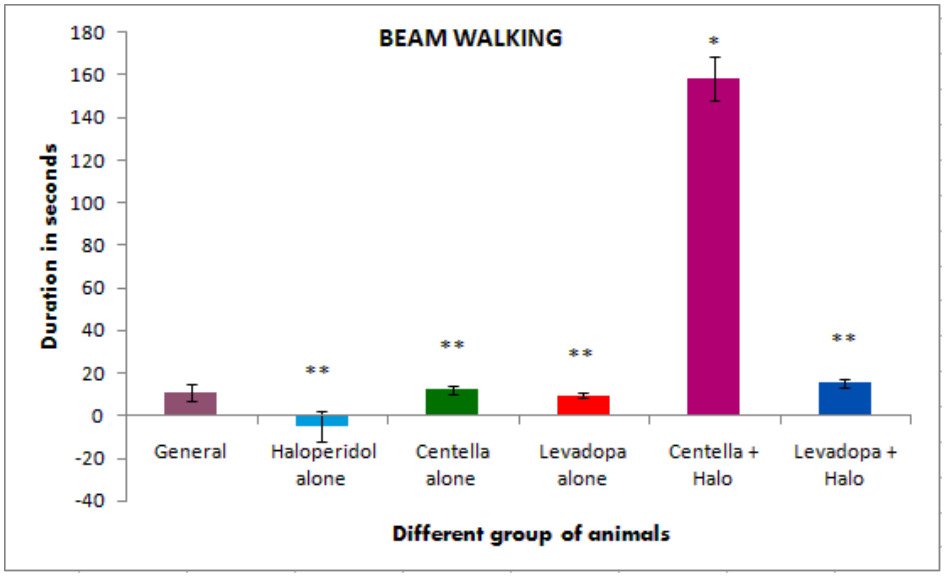
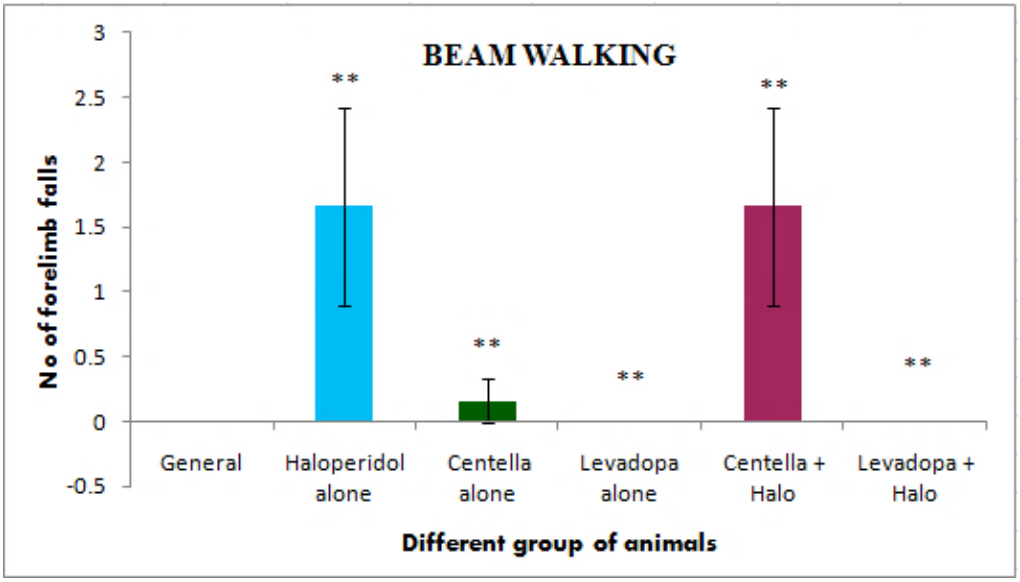


Graph 3: Beam walking flaw scores in crossing duration of different groups of animals (n=6).

****Significant at 1% level.** ***Significant at 5% level.**

The above graph shows the comparisons of the beam walking flaw scores in fore limb of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed a decline in the scoring than other groups. And the test group 1 (haloperidol + *Centella asiatica*) showed increasing level of scoring than the test group 2 (haloperidol + Levodopa



Graph 4: Beam walking flaw scores in hind limb of different groups of animals (n=6).

****Significant at 1% level.** ***Significant at 5% level.**

The above graph shows the comparisons of the beam walking flaw scores in fore limb of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed an increased scorings than the other groups. Test group 1 (haloperidol + *Centella asiatica*) showed a significant increase in the level of scorings than the test group 2 (haloperidol + levodopa).

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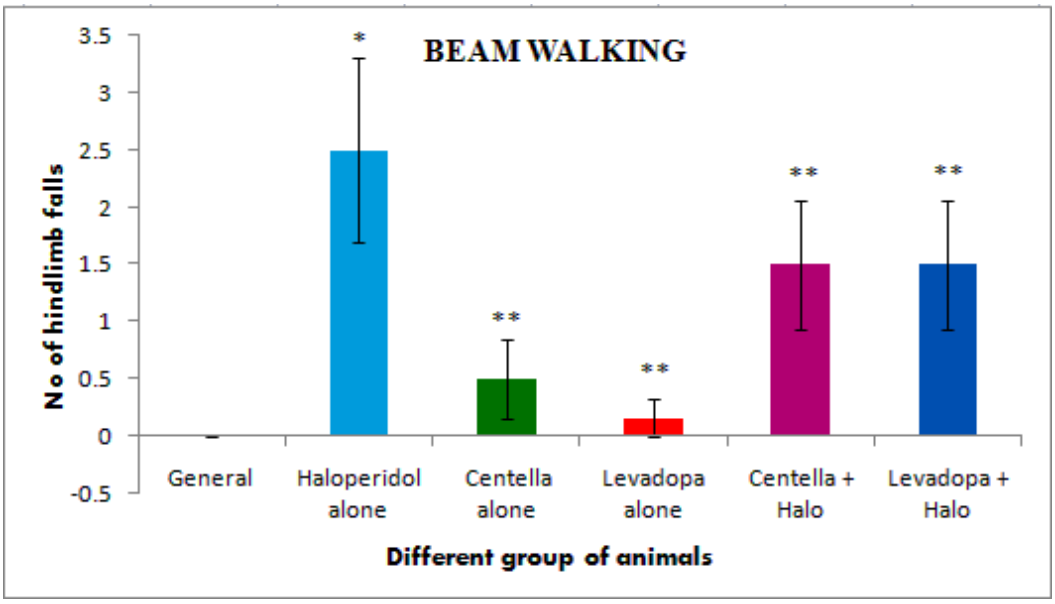
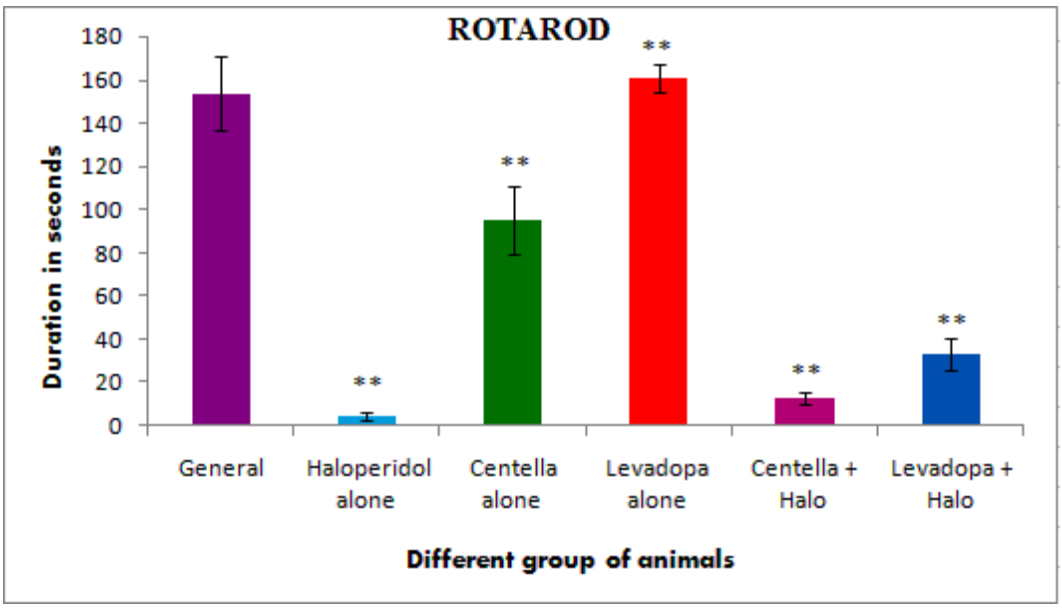


Graph 5: Beam walking flaw scores in hind limb of different group of animals (n=6).

****Significant at 1% level. ***Significant at 5% level.

The above graph shows the comparisons of the beam walking flaw scores in hind limb of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed increased in the scorings than the other groups. And the test group 1 (haloperidol + *Centella asiatica*) showed a significant increased level of scorings which is equal to the test group 2 (haloperidol + levodopa).



Graph 6: Rotarod score of different groups of animal (n=6).

****Significant at 1% level. ***Significant at 5% level.

The above graph shows the comparisons of the rotarod scores of different groups of animals (n=6). From the analysis, control group 1 (haloperidol) showed a decline in the scorings than the other groups. And the test group 1 (haloperidol +*Centella asiatica*) showed a decline in the scorings than the test group 2 (haloperidol + levodopa).

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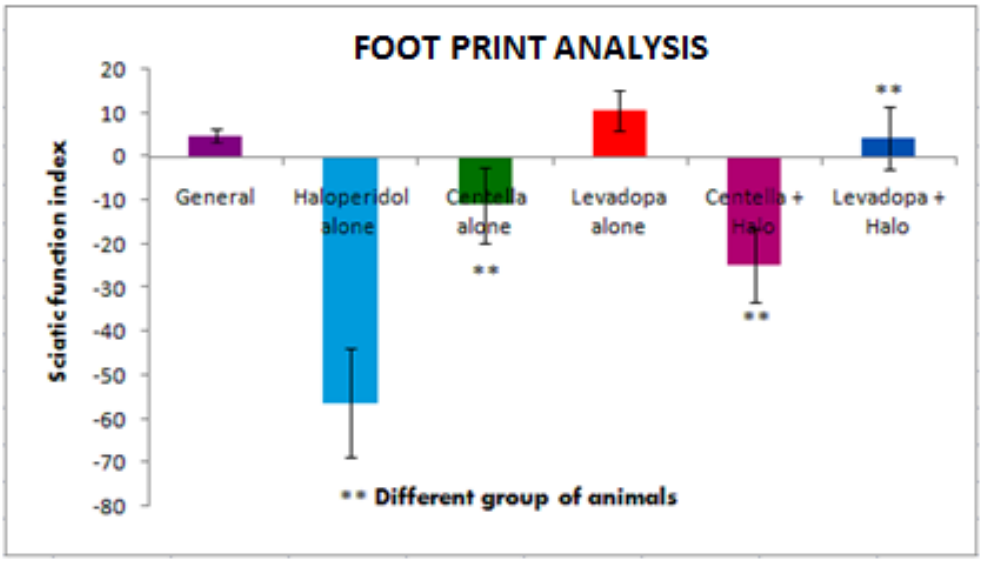

Graph 7: Sciatic function index of different groups of animals (N=6).

****Significant at 1 % level. ***Significant at 5 % level.

The above graph shows the comparison of sciatic function index (SFI) reading of different groups of animals. Before the treatment it was found to be normal. The test and the control groups were injected with haloperidol. After inducing Parkinson's disease, sciatic function index showed a decreased reading in the case of haloperidol induced Parkinson's disease control rats when compared with normal rats.

5. DISCUSSION

Parkinson's Disease (PD) is a chronic, progressive, neurodegenerative disorder characterised by tremor, rigidity and slowness of movements and is associated with progressive neuronal loss of the substantia nigra and other brain structures. Non-motor features, such as dementia and dysautonomia occur frequently, especially in advanced stages of the disease. The pathogenesis of PD is believed to be multifactorial. Both environmental and genetic factors contribute to PD pathogenesis. The disease progresses slowly in most people and the symptoms take years to develop. Although it can be diagnosed accurately, no therapeutic strategies can cure or completely block the progression of PD. Treatment aims at suppressing the symptoms of the disease with the least amount of adverse effects from the drugs. Drug therapy aims to restore the dopaminergic cholinergic balance. Some drugs will increase dopaminergic function and decrease striatal cholinergic activities. The most common drugs used were Dopamine agonists, Anticholinergics Antihistaminics, Monoamine oxidase B (MAO-B) inhibitors and Catechol O-methyl transferase (COMT). Medications, surgery and multidisciplinary management can also provide relief from the symptoms.

Plants have been used as treatments for hundreds of years, based on experience and folk remedies and continue to draw wide attention for their role in the treatment of mild and chronic diseases. *Centella asiatica* is a very important medicinal herb with diverse activities. It is well known for its traditional uses and medicinal properties. *Centella asiatica* commonly known as *Gotu kola*, *Kodangal*, *Karinga*, *Muttil* etc. It contains several active constituents, of which the most important are the triterpenoid saponins, including asiaticoside, centelloside, madecassoside and asiatic acid. In addition, Centella contains other components, including volatile oils, flavonoids, tannins, phytosterols, amino acids and sugars.

This study is to explore the effect of oral administration of aqueous extract of *Centella asiatica* in the management of Parkinsonism. A wide range of behavioral studies were done in rats to analyze different aspects of cognitive and motor functions and this may also help to diagnose neurological disorder symptoms. In the present study various motor experiments scoring analysis show the significance in the management of Parkinsonism.

Open field is a motor experiment used for measuring anxiety and exploration as well as locomotion as it has a large central area. Repeated exposure to the open field apparatus results in time dependent changes in behavior [10]. In the present study the exploratory scoring and rearing patterns in general and test groups were analysed. After inducing PD in

control and test rats, it was seen that the rotarod reading in control group were much lower in comparison with the normal rats. A similar test was conducted on haloperidol induced Parkinsonian rats. It was observed that the haloperidol induced Parkinson's diseased rats had a lower reading as compared with the other groups. The rotarod performance test is based on a rotating rod with forced motor activity. The rotarod performance test evaluates balance and sensory motor coordination of the subjects. It is used to evaluate the alterations in rotarod performance in animal models of pathological situations and compared with control rats.

Beam walking also assesses motor coordination, particularly of the hind limbs. The difficulty of this task can be varied by using beams with different shapes and widths. The time taken to walk along the beam by the control group having Parkinson's disease are higher as compared with the general group. In our test, the PD rats also showed motor impairment and could not walk along the beam. After the treatment with *Centella asiatica*, the test group rats showed somewhat significant reading compared with the normal group and the levodopa treated group showed better effect than the test group 1 (haloperidol + *Centella asiatica*). Along with the beam walking test, the foot drop of both the hind limbs and fore limbs was also observed. The motor dysfunction and gait disorders are initially manifested as lower extremity weakness. Quantitative method of analyzing the sciatic nerve functions in rats is known as the Sciatic Function Index. Many studies used the Sciatic Function Index as an assessment of hind limb function after sciatic nerve lesions and repair, which can be quantified reliably and easily by gait analysis through foot prints. In the present study, the control group showed lower sciatic function index reading when compared with the normal group. After treatment with *Centella asiatica*, the test group showed similarity in fore limb drop readings when compared with the normal group. The levodopa treated group showed better effect than the test group 1 (haloperidol + *Centella asiatica*).

Similar studies were conducted to evaluate the sciatic function index and to assess the motor impairment by walking track analysis, where the animal was placed in a walking pathway ending in a darkened cage. All rats were first allowed two or three conditioning trials, during which they often stop to explore the corridor, thereafter they walk steadily to the darkened cage [11].

An SFI of 0 value is normal. An SFI of -100 indicates total impairment, such as would result from a complete transection of the sciatic nerve [12]. The walking track analysis clearly demonstrated that there is a direct relationship between individual hind limb muscle function and print measurements. After treatment with *Centella* and Levodopa, the test group 2 (Levadopa alone + Haloperidol) showed similarity in the Sciatic Function Index reading when compared with the normal group. This study provides evidence that *Centella asiatica* played a significant role in ameliorating Parkinson's disease symptoms.

The effect of Levadopa is found to be better in all the experiment as the mechanism of its action is highly specific on the neurons as neurotransmitters and the Centella, as it is a crude extract containing a few hundred active molecules, the mode of action may be different from the levadopa mechanism of action.

6. CONCLUSION

The present study reveals that aqueous extract of *Centella asiatica* has a significant role in the management of Parkinson's disease. There is a significant difference in between the control 1 (haloperidol treated) and test 1 (haloperidol + *Centella asiatica* treated) groups. Within test groups, levodopa treated animals performed better than the animals fed with *Centella asiatica*.

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