JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

NEUROPROTECTIVE EFFECTS OF *Withania somnifera* AND *Camellia sinensis* ON 6-OHDA-LESIONED RAT MODEL OF PARKINSON'S DISEASE

Mahendra A. Giri*[1], K. Vanitha Prakash[2], Sanjay B. Kasture[3]

[1]Matoshri institute of Pharmacy, Dhanore, Yeola, Maharashtra, India 423401

[2] Shri Sai Jyoti College of pharmacy, Vattinagula Pally, Gandhipeth, Hydrabad 500075

[3] Pinnacle Biomedical Research Institute, Bhopal, Madhya Pradesh, India 462003

*Corresponding author

Mr. Mahendra A. Giri

Email ID: mahi_jaan83@yahoo.com

Department of Pharmacology,

Matoshri Institute of Pharmacy, Dhanore, Yeola

Maharashtra, India 423401

ABSTRACT

Context: Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting about 1.5% of the global population over 65 years of age. Oxidative stress plays an important role in the pathogenesis of neurodegeneration. *Withania somnifera* and *Camellia sinensis* are traditional herbs known to have neuroprotective and antioxidant effects. In the present study, comparative antiparkinsonian effect of hydroalcoholic extract of *Camellia sinensis* leaves (HECS) and *Withania somnifera* roots (HEWS) on 6-OHDA-Lesioned Rat Model was studied and this experiment evaluated the effect on the hemiparkinsonism induced in rats. The results were analyzed by repeated measure ANOVA followed by Dunnett's test. In this study, the selected doses of HEWS and HECS & their MIXTURE in equal parts at 30mg/kg &100mg/kg exhibited neuroprotective effect. These treatments increased contralateral turing behaviour indicative of antiparkinsonian activity.

Key-words: Antiparkinsonian, Haloperidol, Reserpine, Tacrine, *Withania somnifera*, *Camellia sinensis*

INTRODUCTION

Neurodegenerative diseases like Alzheimer's, Parkinson's, Huntington's and multiple sclerosis are associated with the process of memory loss and cognitive decline which results from selective degeneration of particular neuronal cells and the deposit of aggregated proteins. Parkinson's disease (PD) is mainly characterized as a movement disorder but non-motor symptoms are also involved. Since dopamine is associated with motor activity, the progressive loss of dopaminergic neurons in PD leads to muscle rigidity, tremors and bradykinesia as well as cognition, mental, sleeping, personality and behaviour disorders including depression and anxiety [1-2]. The mechanisms responsible for dopaminergic neuronal loss in PD are complex and yet unclear. Pathogenic factors such as oxidative and nitrosative stress, mitochondrial dysfunction, apoptosis, inflammatory responses and excitotoxicity have been proposed for the degeneration of dopaminergic neurons. Literature review suggests increased reactive oxygen species (ROS) and oxidative damage in the cascade of events leading to degeneration of dopaminergic neurons. This is mainly due to the observations that increased level of lipid peroxidation, modifications of proteins, and DNA and RNA oxidation products are seen in the brain of Parkinsonian patients [3-4]. Currently, there is no cure for PD and the drugs used for treatment are levodopa, dopamine agonists and monoamine oxidase-B (MAO-B) inhibitors, which provide only symptomatic relief. Levodopa has been considered the gold standard drug therapy for Parkinson's disease but it is limited only to relieving symptoms and its long term use may cause serious side effects that include involuntary movements (dyskinesia), the on-off effect may cause Parkinson's related movement problems to appear and disappear suddenly and unpredictably. The side effects of allopathic medicines for PD are highly alarming; hence, the current research is now focusing on herbs used in alternative systems of medicine as neuroprotective [5]. In this quest, some herbs have been found to be effective neuroprotectants. Phytoconstituents like polyphenols, flavonoids exhibit antiparkinsonian activity against experimentally induced PD. *Withania somnifera* and *Camellia sinensis* are an important plants used in Ayurveda for the treatment of various disorders of the CNS and are rich in polyphenols, flavonoids, alkaloids and lactones. *Camellia sinensis* is popularly known as Green Tea belonging to Theaceae family. The most important phytoconstituents of *Camellia sinensis* are polyphenolic compounds known as catechins including epigallocatechin gallate (EGCG), catechin (C), epicatechin (EC), gallocatechin (GC), gallocatechin gallate (GCG), epigallocatechin (EGC), and epicatechin gallate (ECG). Flavonols contribute to the antioxidant capabilities of tea leaves. The aglycones of the main flavonols in tea leaves are quercetin, kaempferol, and myricetin. Pharmacologically active constituents of *Camellia sinensis* have been shown to possess hepatoprotective, cardioprotective, neuroprotective, anticancer, antiobesity, antidiabetic, antibacterial, antiviral and antioxidant effects. Antioxidant property of catechin contributes to protection from neurodegeneration [6-7]. *Withania somnifera* commonly known as Ashwagandha, Asgand,

Indian ginseng, and winter cherry belongs to the family Solanaceae is an important medicinal plant that has been used in Ayurvedic and indigenous medicine. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X). *Withania somnifera* is used to calm the mind, relieve weakness and nervous exhaustion, build sexual energy and promote healthy sleep. The herb is termed a rasayana, means it acts as a tonic for vitality and longevity. Numerous studies indicated that *Withania somnifera* possesses antioxidant, antitumor, antistress, anti-inflammatory, immunomodulatory, hematopoietic, anti-ageing, anxiolytic, antidepressive, rejuvenating properties and also influences various neurotransmitter receptors in the central nervous system [8-9]. This experimental study was done to evaluate the neuroprotective activity of the Hydroalcoholic extracts of these two plants, *Withania somnifera* and *Camellia sinesis*, with 6-OHDA-Lesioned Rat Model with a view that these plant extracts shall have no or at least reduced adverse effect so that it can be used for long duration.

MATERIAL AND METHOD

Experimental Animals

Male Sprague–Dawley rats weighing 250–400 g were divided in different groups in polycarbonate cages (width x length x height 33 x 56 x 20 cm). Each group contained 6 animals.

Drugs and Chemicals

6-OHDA & benserazide were purchased from Sigma Aldrich. The extract HECS, HEWS & Mixture of each extract in equal proportion in doses of 30 mg/kg & 100 mg/kg body weight of rats.

Plant material and extraction

Dry powder of *Withania somnifera* roots and *Camellia sinensis* leaves was purchased from local market and were authenticated from department of Pharmacognosy, Sanjivani College of Pharmaceutical Education and Research, Kopargaon.

Hydroalcoholic extracts were prepared using Soxhlet's extractor. The extracts were filtered and dried. Extracts were subjected to phytochemical screening [10]. The extracts were administered in doses of 30 and 100 mg/kg (p.o.). Control group was given only vehicle in equivalent volume of plant extract.

Determination of anti-parkinsonian activity by using 6-OHDA model

6-OHDA Lesioning

As per the method given by Atlas of Pellegrino et al. (1979) animals were anesthetized with chloral hydrate (450 mg/kg i.p.). Using stereotaxic apparatus, rats were infused with 6-OHDA–HCl (8µg/4µl saline containing 0.5% Ascorbic acid) into the left medial forebrain bundle at coordinates given in method (A=−2.2, L=+1.5, V=−7.8), at a rate of 1µl/min and the needle was removed 2 min after complete injection.

Pre-treatment with desipramine (at a dose of 10 mg/kg i.p.) 30 min before 6-OHDA was given to all animals to avoid impairment of noradrenergic neurons.

Assessment by using Stepping Tests

For making animals familiarize with the experimenter's grip they were handled for the first 3 days as described below, 2 weeks before 6- OHDA lesion. Training to run spontaneously up the ramp 1m long in the direction of the home cage was given over the next two days. After lesion in the fourth week, animals were divided into eight groups & treated with benserazide (6 mg/kg i.p.) plus levodopa (6 mg/kg) or HECS, HEWS & Mixture of all extracts in equal quantitiy, in 30 mg/kg & 100 mg/kg oral dose were used.

Initiation time assessment:

With one hand the rat was held in such a way that only one forepaw was available for the movement. The rat was slightly lifted holding the hind part of the rat body above the ramp surface. Free forelimb was placed on the ramp till the rat initiated the step and thus initiation time was measured. The initiation time was thus the time lapsed between placing the forepaw on the ramp and initiating the first step.

Step adjustment assessment:

Rats were held in the same position as in the above test with their forelimbs touching the table (0.7 m long). Rats were slowly moved laterally along the table surface, first forward and then backward (0.7 m in 4 seconds). In both directions the number of adjusting steps taken by the right & left forelimbs was measured. The test sequence was initially right forelimb & then left forelimb in forward & backward adjusting steps. Rats were tested two times in a day for a 3-day, one week before & two, three weeks after the 6-OHDA lesion to assess for stepping & adjusting test. Therefore, all rats were tested for baseline values (pre-lesion test) and retested after 6-OHDA lesions to obtain values for lesion-induced defects. Then, rats were randomly sub-divided into eight groups and treatment with HECS, HEWS & Mixture in equal proportion was given. After drug administration adjusting & stepping tests were performed at 2 different times based on the drug's pharmacological effect onset (initial test 15 min after HECS, HEWS & Mixture) & full pharmacological effectiveness (subsequent test 45 min after HECS, HEWS & Mixture). Testing sequence was right forelimb and then left forelimb which was repeated twice [11,12].

Vibrissae-Elicited Forelimb Placing

The vibrissae of the rat are the sensiromotor organs and upon touching the vibrissae, the rat moves up the ipsilateral forelimb. The rat was held at the chest, letting forelimbs to hang free, & against the edge of the table top brushed its vibrissae to produce a forelimb placing response from the forelimb on the same side. Placement was quantified as a percentage of successful placement responses obtained from 10 subtests. The subtests that rats struggled with were not counted. Rats were trained on a placing test 2 weeks prior to surgery (10 trials for each forelimb daily). To promote muscle relaxation and eliminate combative movements, the experimenter made gentle up and down movements in space before assessing the placement response [13].

Assessment of Turning Behavior

Turning behavior was evaluated in a hemispherical bowl with sawdust on the floor and complete (360°) rotations in both directions (ipsilateral and contralateral i.e. opposite to the injured hemisphere) were recorded. An injection of benserazide (15 mg/kg i.p.) plus levodopa or HECS, HEWS & mixture in equal proportion (30 mg/kg & 100 mg/kg) was given to rats 2 weeks after 6-OHDA-infusion. Rats those displayed at least 300 contralateral turning during the 2 h testing period were kept in the study. Rats were separated into different groups (receiving levodopa, HECS, HEWS & mixture in equal proportion, respectively, on the basis of HECS, HEWS & mixture in equal proportion and contralateral turning behaviour was recorded in the all groups. 30 min before drug administration rats were placed in the observation bowls to adjust & extinguish any spontaneous rotational behaviour [11,12].

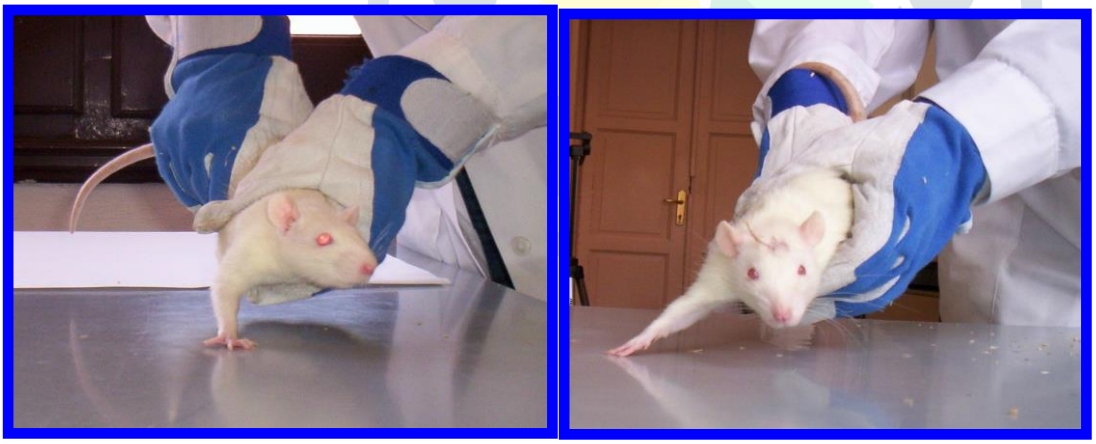
RESULTS

6-OHDA Induced Parkinsonism In Rats:

Stepping Test

Unilateral 6-OHDA-lesioned rats displayed a significantly impaired motor performance of the right forelimb, contralateral to the 6-OHDA lesion, in the stepping test, as compared to the pre-lesion test (Figure no. 2). As expected and reported earlier, no impairment was observed in the movements of left forelimb.

Figure no. 1: Stepping test



Before 6-OHDA lesion **After 6-OHDA lesion**

Assessment of Step Initiation

Latency of step initiation, progressively increased with the right forelimb ($p < 0.0001$ vs. pre-6-OHDA lesion) from 2 to 3 weeks post-lesion ($p < 0.0001$ vs. pre-6-OHDA lesion) (figure no. 2) Four weeks after lesion, rats receiving mixture at 30 mg/kg i.p. HECS, HEWS & mixture in equal proportion displayed a reversal of motor impairment of right forelimb in the initiation time at both 15th and 45th min but when mixture dose was increased it also reversed motor impairment but was less significant as compared 30mg/kg i.p dose of mixture alone.

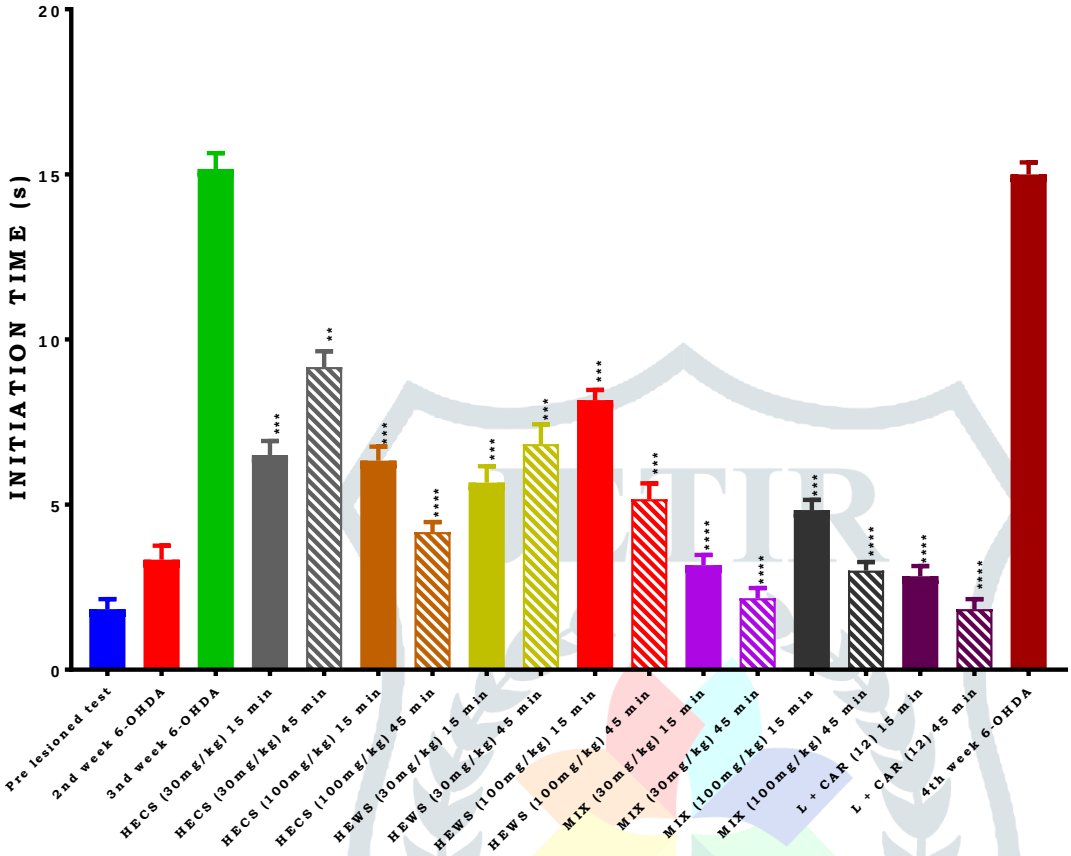


Figure no. 2: Assessment of initiation time Right forelimb for HECS, HEWS and MIXTURE in equal quantitiy at 30mg/kg and 100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

Assessment of Adjusting Step

6-OHDA lesion induced a significant deficit in the number of adjusting steps when rat was moved forward and backward by the experimenter (Figure No. 3 and 4). Administration of mixture at a dose of 30 mg/kg effectively reversed the effect of the lesion as shown by improving the number of steps both in the forward and backward direction except HECS (30 mg/kg) at 15 min interval (fig No. 4). At a dose of 100 mg/kg, the mixture also significantly improved performance of the right forelimb in forward and backward direction but to the lesser extent as compared to 30 mg/kg dose.

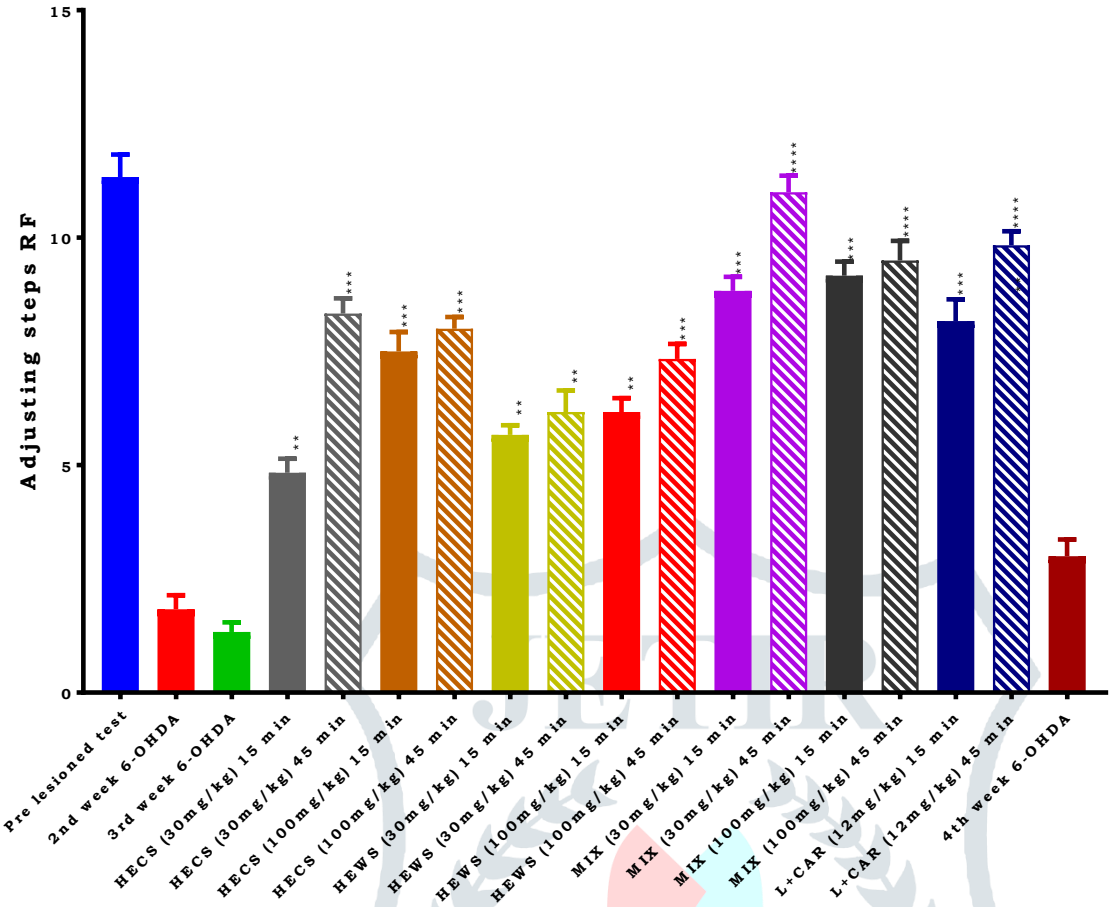


Figure no. 3: Adjusting steps: forward Right forelimb for HECS, HEWS & MIXTURE (1:1:1:1:1) at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

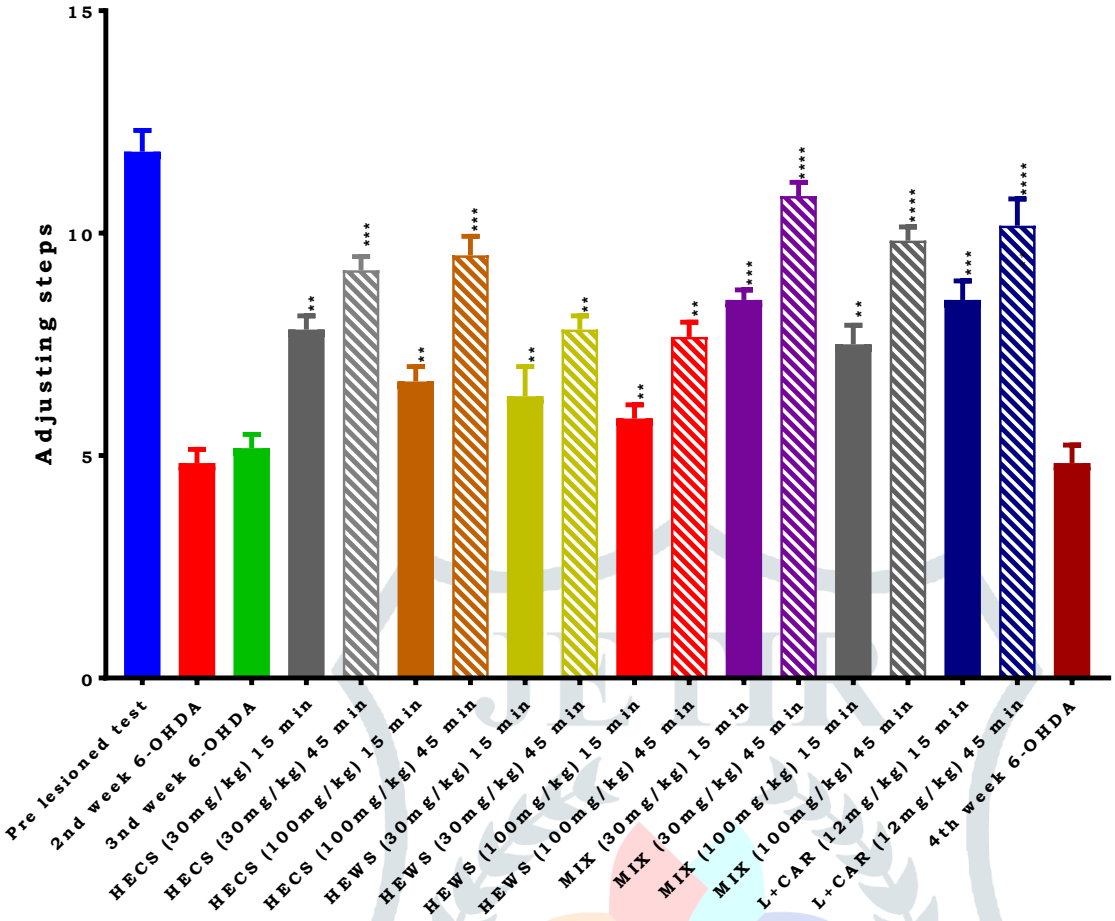


Figure no. 4: Adjusting steps: Backward Right forelimb for HECS, HEWS & MIXTURE (1:1:1:1:1) at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

Vibrissae-Elicited Forelimb Placing

Rats tested 2, 3, and 4 weeks after 6-OHDA lesion was showing extremely significant reduction in percent response for Vibrissae elicited forelimb placing test means after vibrissae-brushing lesioned rats did not place right forelimb on the table as compared with pre lesioned test for right forelimb (figure no. 5). As compared to the 2nd and 3rd week, the lesioned rats on 4th week did not improve placement of the right forelimb, in contrast, at a dose of 30 mg/kg and 100 mg/kg, HECS, HEWS and mixture significantly restored forelimb placing performance. Combination displayed confirmed effectiveness in restoring placement of the right forelimb at a dose of both 30 mg/kg and 100 mg/kg (Figure no. 5)

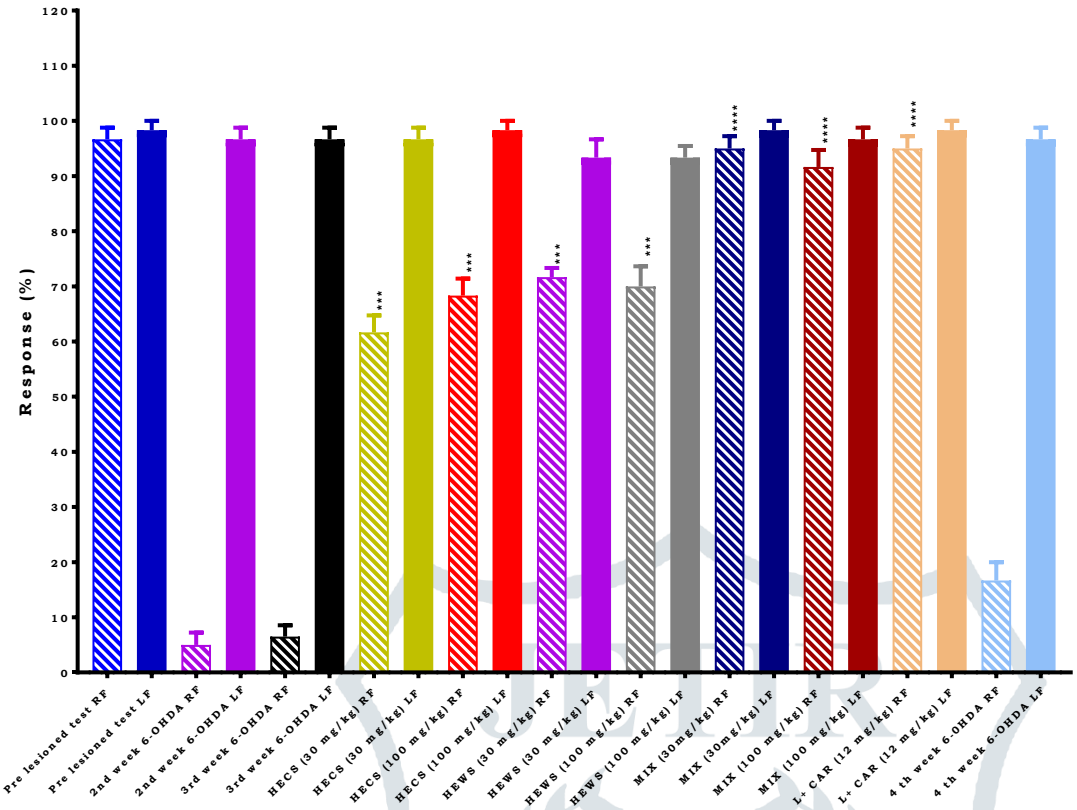


Figure no 5: Vibrissae-Elicited forelimb placing for HECS, HEWS & MIXTURE (1:1:1:1:1) at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

Assessment of Turning Behaviour

As compared to the control group, all extracts showed significant increase in contralateral turnings at 30 mg/kg dose (figure no. 6). In comparison with control group, 100 mg/kg dose of HECS, HEWS and mixture showed significant improvement in contralateral turning behavior (figure no. 6 and 7).

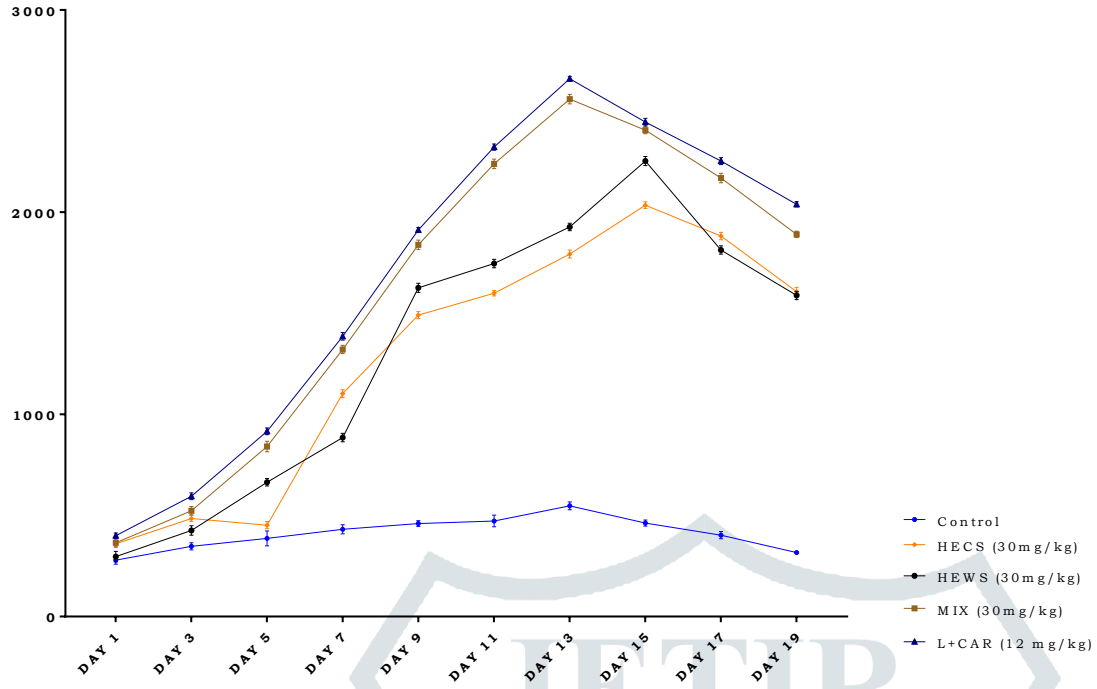


Figure no. 6: Assessment of contralateral turning behaviour after HECS, HEWS & MIXTURE (1:1:1:1:1) at 30 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

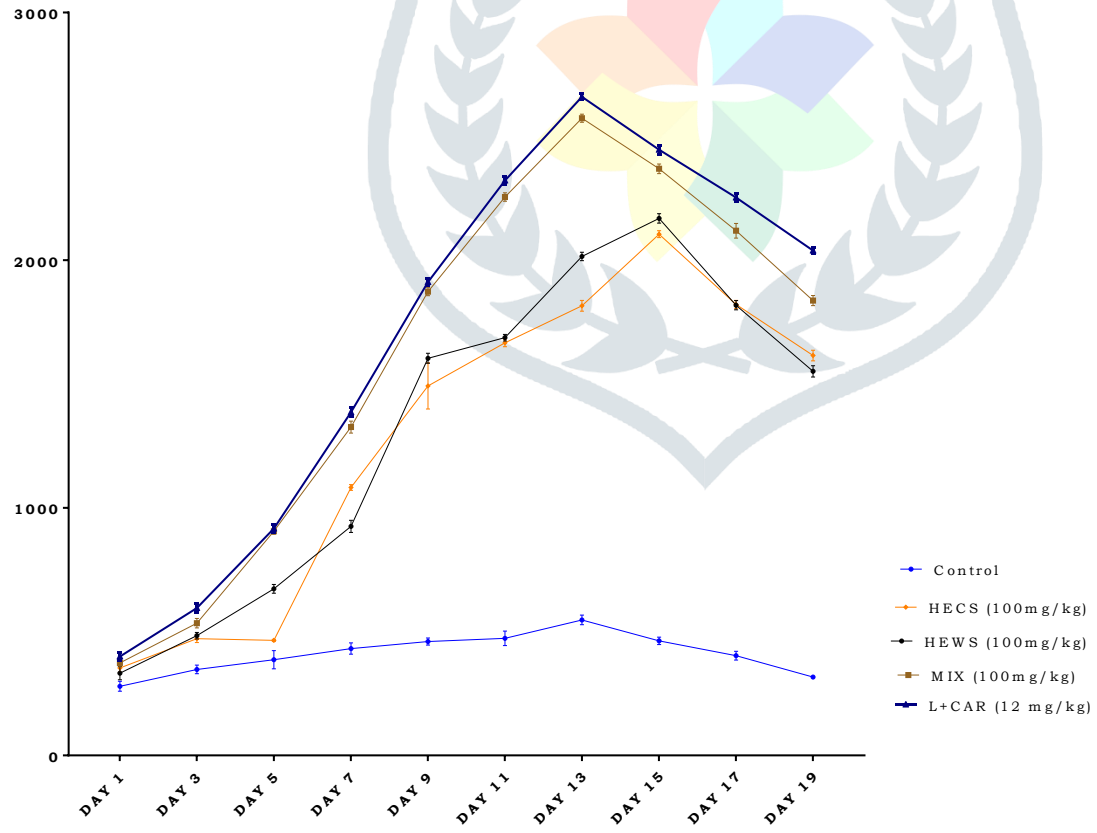


Figure no. 7: Assessment of contralateral turning behaviour for HECS, HEWS & MIXTURE (1:1:1:1:1) at 100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

DISCUSSION

6-OHDA INDUCED ANTIPARKINSONIAN ACTIVITY:

In contrast to the previous set of experiments, this experiment evaluated the effect on the hemiparkinsonism induced in rats. The previous experiments evaluated the preventive effect whereas this experiment evaluated the curative effect in the animal models. In this study, the selected doses of, HECS, HEWS & their MIXTURE in equal parts at 30mg/kg &100mg/kg exhibited neuroprotective effect. These treatments increased contralateral turing behavious indicative of antiparkinsonian activity [11]. Unilaterally 6-OHDA-lesioned rats manifest a marked sensory-motor integration deficit modeled by the vibrissae-evoked forelimb placing [13, 14].

REFERENCE:

1. Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Lanciego JL, Artieda J, Gonzalo N. Pathophysiology of the basal ganglia in Parkinson's disease. Trends Neurosci 2000;23(10):18-9.
2. Kasture S, Pontis S, Pinna A, Schintu N, Spina L, Longoni R, et al. Assessment of symptomatic and neuroprotective efficacy of Mucuna pruriens seed extract in rodent model of Parkinson's disease. Neurotox Res 2009;15(2):111-22.
3. Baluchnejadmojarad T, Rabiee N, Zabihnejad S, Roghani M. Ellagic acid exerts protective effect in intrastriatal 6-hydroxydopamine rat model of Parkinson's disease: Possible involvement of ERβ/Nrf2/HO-1 signaling. Brain Res 2017;1662:23-30.
4. Ahmad M, Saleem S, Ahmad AS, Yousuf S, Ansari MA, Khan MB, et al. Ginkgo biloba affords dose-dependent protection against 6-hydroxydopamine-induced parkinsonism in rats: Neurobehavioural, neurochemical and immunohistochemical evidences. J Neurochem 2005; 93(1):94-104.
5. Ittiyavirah SP, Ruby R. Effect of hydro-alcoholic root extract of plumbago zeylanica l alone and its combination with aqueous leaf extract of camellia sinensis on haloperidol induced parkinsonism in wistar rats. Ann Neurosci 2014; 21(2): 47–50.
6. Bedrood Z, Rameshrad M, Hosseinzadeh H. Toxicological effects of Camellia sinensis (green tea): A review. Phytother Res 2018;32(7):1163-80.
7. Williams RJ, Spencer JPE. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med 2012. 52(1):35-45.

8. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific & Industrial Research, New Delhi (1980) 191, 258.
9. Dhuley JN. Effect of ashwagandha on lipid peroxidation in stress-induced animals. J Ethnopharmacol 2019;60(2):173–8.
10. Hamdy E. Trease and Evans Pharmacognosy. 16th edition, Sunders, London, 2009; 121-35.
11. Kasture S, Pontis S, Pinna A, Schintu N, Spina L, Longoni R, Simola N, Ballero M, Morelli M. Assessment of symptomatic and neuroprotective efficacy of Mucuna pruriens seed extract in rodent model of Parkinson's disease. Neurotox Res. 2009 Feb;15(2):111-22.
12. Pinna A, Pontis S, Borsini F, Morelli M. Adenosine A2A receptor antagonists improve deficits in initiation of movement and sensory motor integration in the unilateral 6-hydroxydopamine rat model of Parkinson's disease. Synapse. 2007 Aug;61(8):606-14.
13. Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology. 2000 Mar 3;39(5):777-87.
14. Meredith GE, Kang UJ. Behavioral models of Parkinson's disease in rodents: a new look at an old problem. Mov Disord. 2006 Oct;21(10):1595-606.