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ANTIPARKINSONIAN EFFECTS OF *Nardostachys jatamansi* ON 6-OHDA-LESIONED RAT MODEL

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ABSTRACT

Parkinsons Disease is the most common progressive neurodegenerative movement disorder affecting more than 10 million people worldwide and the incidence of PD increases with the age. The degeneration of dopaminergic neurons is considered to be the root cause of the characteristic classical motor symptoms and nonmotor symptoms. Oxidative stress plays an important role in the pathogenesis of neurodegeneration. *Nardostachys jatamansi* are traditional herbs known to have neuroprotective and antioxidant effects. In the present study, comparative antiparkinsonian effect of hydroalcoholic extract of *Nardostachys jatamansi* (HENJ) 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 HENJ at 30mg/kg &100mg/kg exhibited neuroprotective effect. These treatments increased contralateral turing behaviour indicative of antiparkinsonian activity.

Key-words: Antiparkinsonian, 6-OHDA, *Nardostachys jatamansi*

INTRODUCTION

In neurodegenerative diseases Parkinsons Disease is the second most common age related disorder which affects more than 10 million people worldwide. Rate of diagnosis of Parkinsonism disease increases with age from that 4 percent of Parkinsons disease are diagnosed before age 50 and in peoples older than age 80 it is stable [1]. Neuronal death in substantial nigra, mitochondrial respiratory failure and increased oxidative stress are the common manifestations in Parkinsons disease [2]. Dopamine neuron degeneration in substantia nigra pars compacta region causes abnormal activities of dopamine within basal ganglia circuits results in muscle rigidity and catalepsy which is the sign of Parkinsons disease [3]. When the nigrostriatal dopamine system activity is reduced causes dystonia, one of the important features of Parkinsons Disease, dopamine receptor blockers can cause acute and tardive dystonia and drug induced parkinsonism [4]. Due to dysregulation in sensory system, pain starting from central to peripheral including polyneuropathy is a heterogeneous symptom in parkinsons disease [5]. In parkinsons disease tremors, bradykinesia, stiffness of the limbs and torso, and postural instability are the four main symptoms from which at least two need to present for epidemiological study of parkinsons disease. Due to dopaminergic cell degeneration excessive activation of ionotropic Glu receptors causes overexcitement of high concentration of Glu and damage neurons called as excitotoxicity, microglial activation, oxidative stress, neuroinflammation and mitochondrial dysfunction leads to apoptosis [6, 7].

Nardostachys jatamansi (NJ) commonly named as jatamansi belongs to family Valerianaceae shows presence of terpenes, saponins, glycosides, flavonoids, tannins and phenolic compounds which are responsible for synergistic reduction in oxidative stress via inhibition of mono-amine oxidase enzymes [8]. Sequiterpines, mainly Jatamansone, and coumarins are main active constituents present in NJ including other sequiterpines such as Alpha-patcho-ulense, Beta- eudesemo, beta-sitosterol, elemol, angelicin, jatamansin, jatamansinol, calarene, beta-atchoulense, n-hexaco-sanyl, n-hexacosane, Oroselol, valeranal, valeranone, seychelane, nardostachnol, nardostachone and also volatile oil [9-10]. Traditionally Nardostachys jatamansi used for tonic, stimulant, and antiseptic effect purpose and have antibacterial, antifungal, antiviral, antioxidant potentials alongside used in nervy headache, menopausal symptoms, flatulence, epilepsy, hyperlipidemia and intestinal colic [10]. This experimental study was done to evaluate the neuroprotective activity of the Hydroalcoholic extract of *Nardostachys jatamansi* with 6-OHDA-Lesioned Rat Model with a view that this plant extract 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 5 animals.

Drugs and Chemicals

6-OHDA & benserazide were purchased from Sigma Aldrich. The extract HENJ is given at doses of 30 mg/kg & 100 mg/kg body weight of rats.

Plant material and extraction

Dry powder of *Nardostachys jatamansi* was purchased from local market and was authenticated from department of Pharmacognosy, Sanjivani College of Pharmaceutical Education and Research, Kopargaon.

Hydroalcoholic extract was prepared using Soxhlet's extractor. The extract were filtered and dried. Extract were subjected to phytochemical screening [11]. The extract was administered at 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 groups & treated with benserazide (6 mg/kg i.p.) plus levodopa (6 mg/kg) or HENJ at 30 mg/kg & 100 mg/kg oral dose.

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 groups and treatment with HENJ 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 HENJ) & full pharmacological effectiveness (subsequent test 45 min after HENJ). Testing sequence was right forelimb and then left forelimb which was repeated twice [12-13].

Vibrissae-Elicited Forelimb Placing

The vibrissae of the rat are the sensorimotor 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 [14].

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 HENJ (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, HENJ). 30 min before drug administration rats were placed in the observation bowls to adjust & extinguish any spontaneous rotational behaviour [14-15].

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). HENJ at 30 mg/kg i.p. was less effective in counteracting impairment as compared to 100 mg/kg i.p.

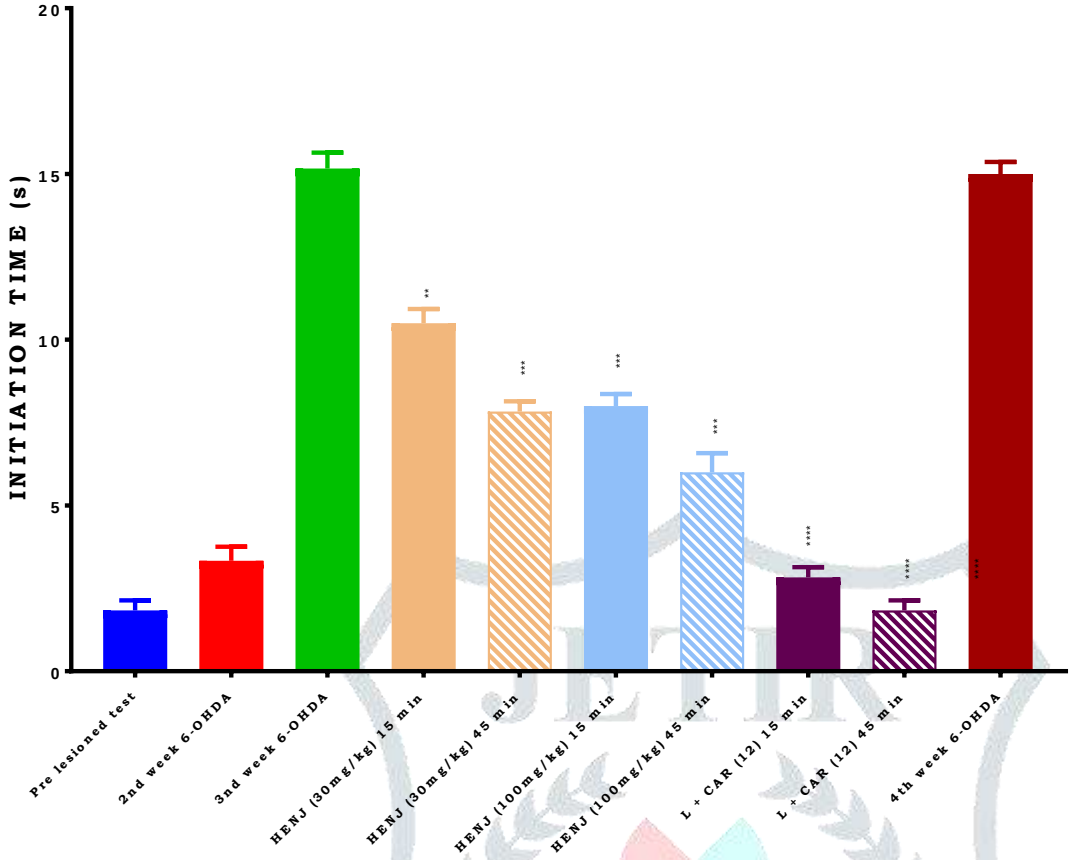

Figure no. 2: Assessment of initiation time Right forelimb for HENJ at 30mg/kg and 100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

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 (Fig No. 3 and 4). Evaluation of performance following HENJ extract 100 mg/kg was also comparable.

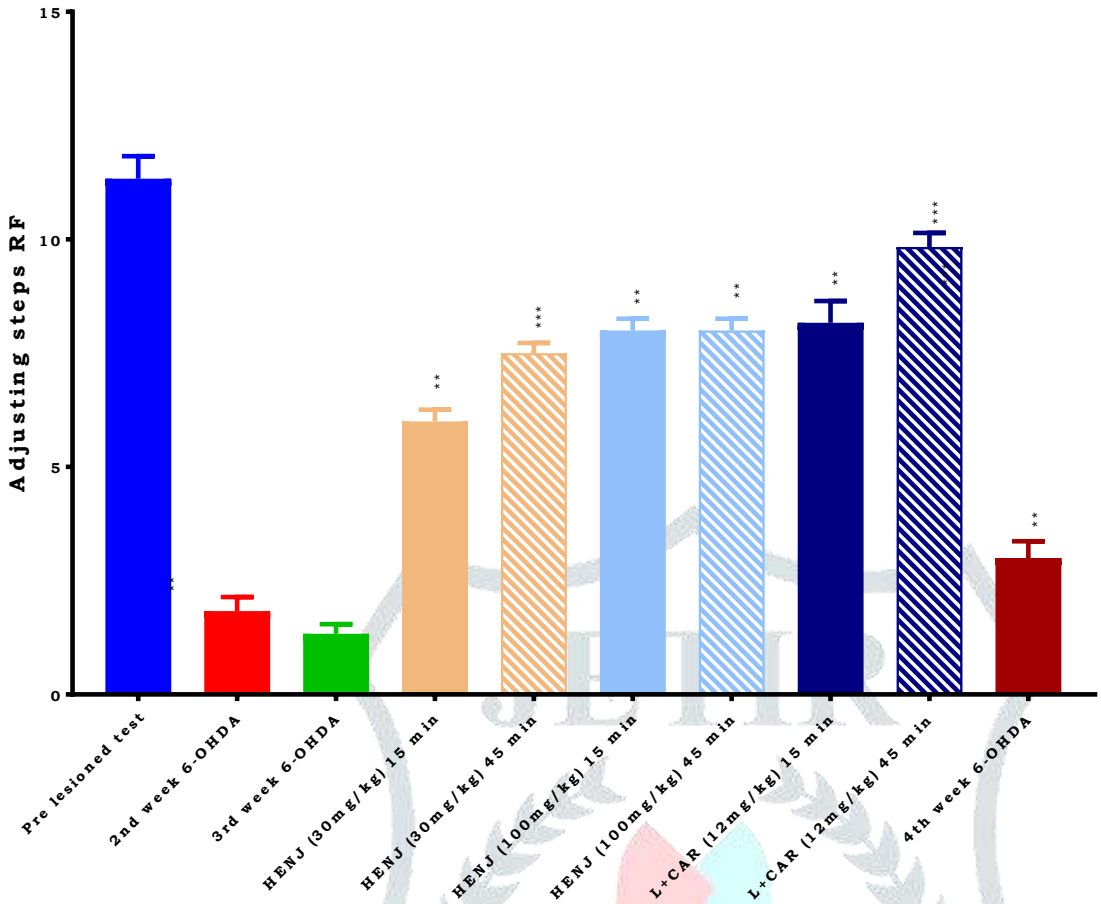

Figure no. 3: Adjusting steps: forward Right forelimb for HENJ at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

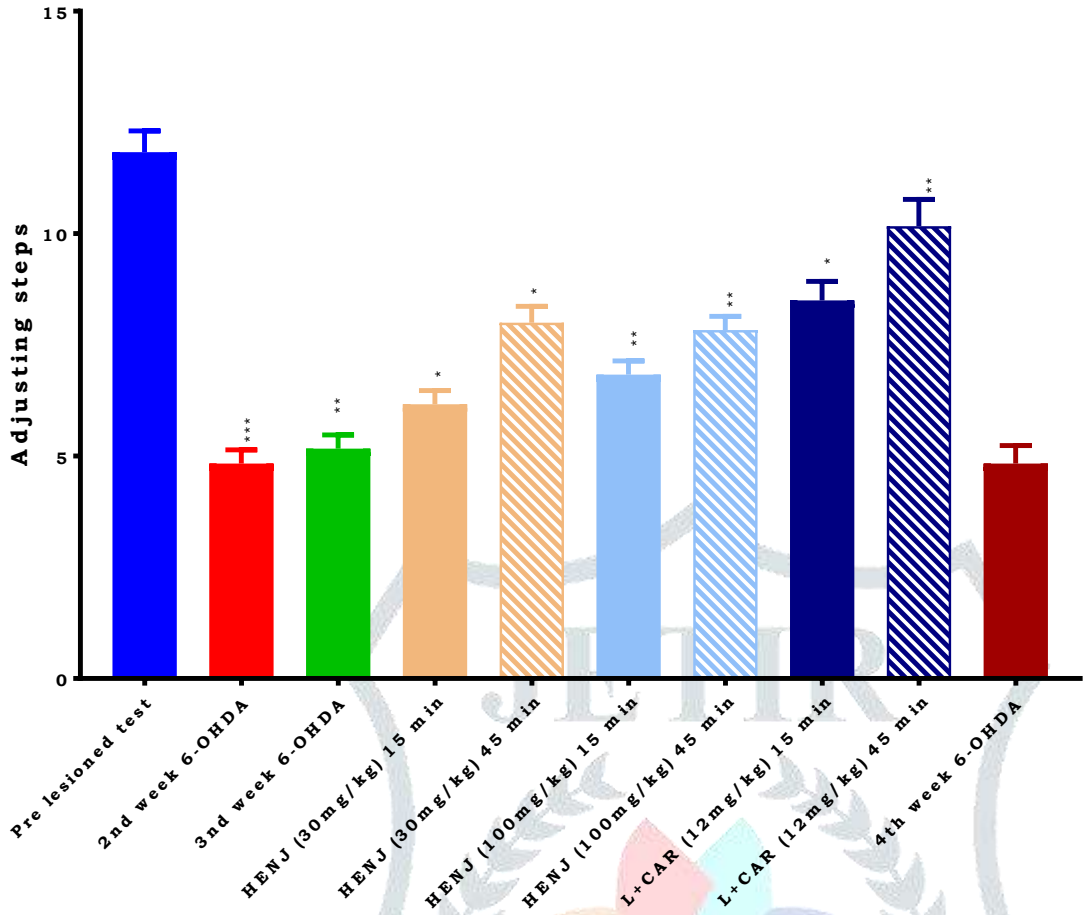

Figure no. 4: Adjusting steps: Backward Right forelimb for HENJ at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

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, HENJ significantly restored forelimb placing performance.

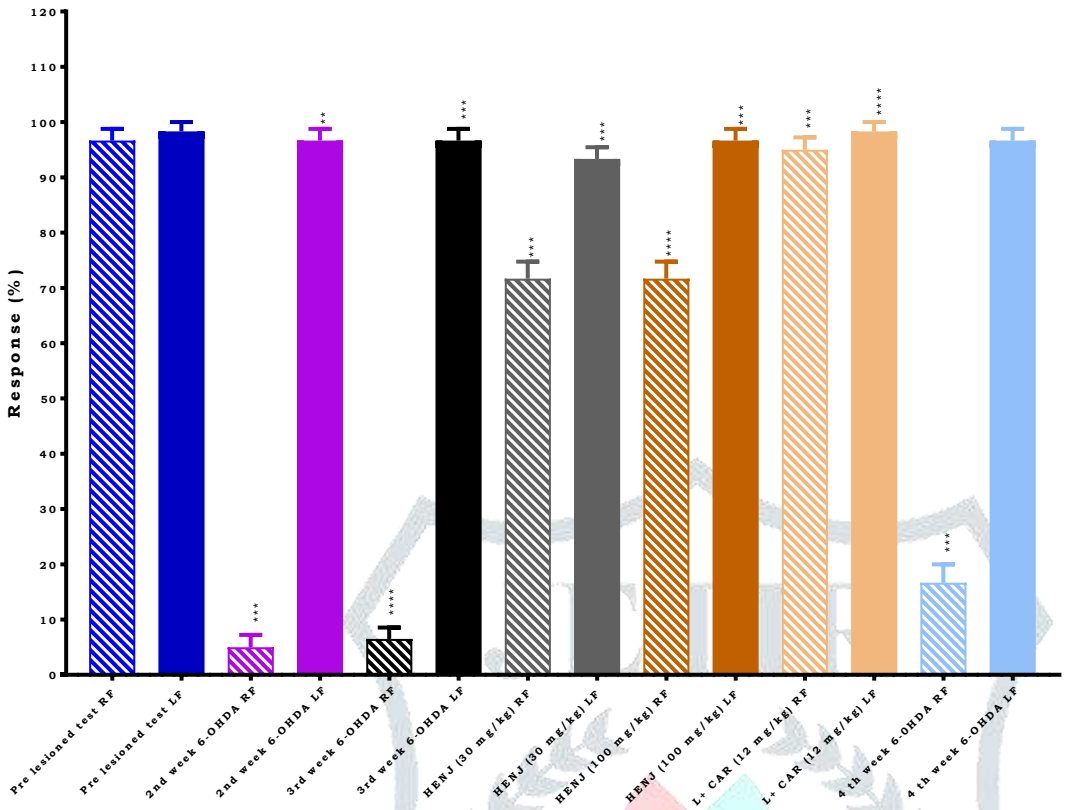

Figure no 5: Vibrissae-Elicited forelimb placing for HENJ at 30mg/kg &100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

Assessment of Turning Behaviour

As compared to the control group, HENJ showed significant increase in contralateral turnings at 30 mg/kg dose (figure no. 6). Day wise improvement was seen in turning behavior and highest performance was recorded on day 13 (figure no.6 & 7).

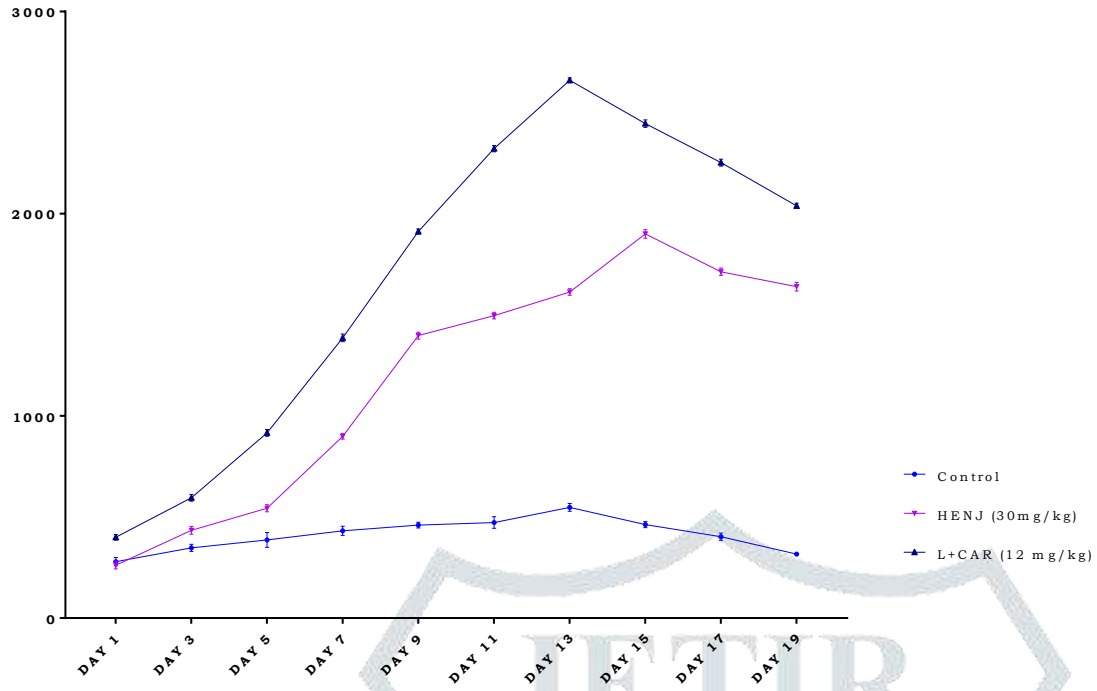

Figure no. 6: Assessment of contralateral turning behaviour after HENJ at 30 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

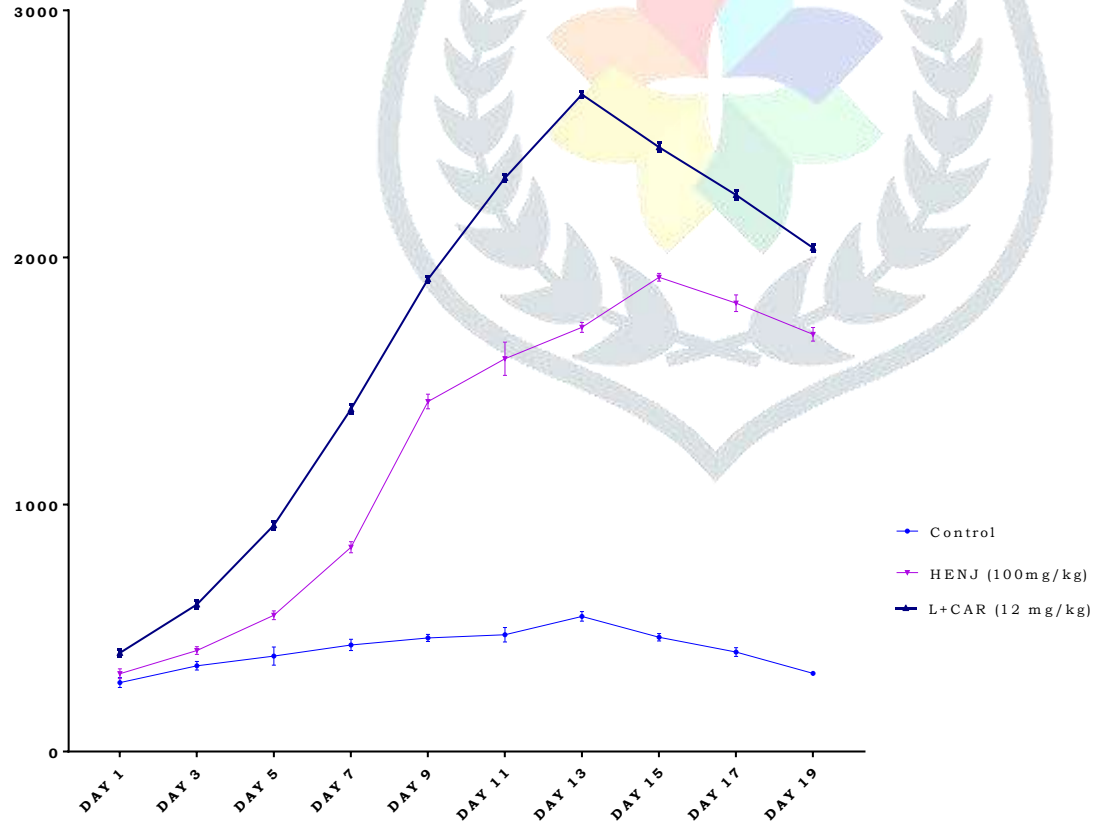

Figure no. 7: Assessment of contralateral turning behaviour for HENJ at 100 mg/kg. All the values are expressed as mean ± SEM; n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

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, HENJ at 30mg/kg &100mg/kg exhibited neuroprotective effect. These treatments increased contralateral turing behavious indicative of antiparkinsonian activity. Unilaterally 6-OHDA-lesioned rats manifest a marked sensory-motor integration deficit modeled by the vibrissae-evoked forelimb placing [14-15].

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