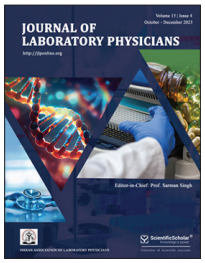
https://jlabphy.org/

Journal of Laboratory Physicians

Article in Press



Original Article

Influence of L-3, 4-dihydroxyphenylalanine on locomotor activities and behavioral changes in rats

Kandra Nagavishnu[1], Karanam Sita Kumari[2], Praveen Kumar Uppala[3], Varri Srinivasa Rao[4], Lingampalli Harika[4], Devu Swathi[5], Sushma Chetan Zilpe[6], Edhi Sandhya[5], Ch.Bhuvan Chandar[5]

[1]Department of Pharmacology, Santhiram Medical College and General Hospital, Nandyal, Departments of [2]Pharmaceutical Biotechnology, [3]Pharmacology, [4]Pharmaceutical Analysis, [5]Pharmacy, [6]Quality Assurance, Maharajah's College of Pharmacy, Vizianagaram, Andhra Pradesh, India.

***Corresponding author:**
Praveen Kumar Uppala, Department of Pharmacology, Maharajah's College of Pharmacy, Vizianagaram, Andhra Pradesh, India.

praveen.chintu32@gmail.com

Received: 03 July 2024
Accepted: 10 October 2024
EPub Ahead of Print: 30 November 2024
Published:

DOI
10.25259/JLP_137_2024

Quick Response Code:

ABSTRACT

Objectives: The central nervous system, the kidneys, the heart, and the hormones are all greatly impacted by dopamine (DA), a neurotransmitter and one of the most significant catecholamines. The goal of this study is to determine if L-DOPA (l-3, 4-dihydroxyphenylalanine) causes any changes in rat behaviour, such as anxiety or motor activity.

Materials and Methods: Before administering L-DOPA intraventricularly, male rats with and without 6-hydroxydopamine (6-OHDA) or oxidopamine lesions were pretreated with benserazide. We then recorded any behavioral changes that occurred with different doses. The rats were placed in a locomotor room, and their movements were recorded to detect changes in locomotor activity.

Statistical analysis: Data were analyzed using Statistical Parametric Mapping and the chi-square test for discrete variables was used to investigate the relationships between DA intensity (O) and behavioural changes. A p-value less than 0.05 were considered statistically significant.

Results: Results demonstrate that 6-OHDA lesioned rats showed quick behavioral changes in response to L-DOPA, in contrast to normally behaving rats that required 3–4 min. Centre and vertical locomotor chamber movements were reduced in 6-OHDA lesion animals compared to normal rats. The entrances to the chamber's center ($F = 23.88$, $P < 0.05$) and vertical motions inside the center ($F = 22.27$, $P < 0.05$) were both significantly impacted by the lesion.

Conclusions: The experimental results conclude that L-DOPA directly elicits changes in the behavioral and locomotor activities of rats. While treating rats with L-DOPA may not improve non-motor functions such as anxiety and depression, it does influence 5-hydroxytryptamine and norepinephrine levels. Consequently, further research into L-DOPA's impacts is needed to identify potential therapeutic targets for the betterment of Parkinson's disease patients' quality of life.

Keywords: L-3, 4-dihydroxyphenylalanine, 6-hydroxydopamine rats, Behavioral changes, Locomotor activities, Dopamine

INTRODUCTION

Dopamine (DA) is an important neurotransmitter and a major catecholamine that plays a role in several physiological functions, such as those of the kidneys, the heart, and the hormones. "Parkinson's disease (PD) is caused by degeneration of dopaminergic neurons in the substantia nigra," one of many neurological illnesses associated with dysregulation of dopaminergic pathways.[1] The striatum's DA levels drop dramatically as a result of this degeneration, which,

in turn, causes motor dysfunction and a host of non-motor symptoms.[2,3]

An essential neurotransmitter and key participant in the best way to alleviate, however, the effects of l-3, 4-dihydroxyphenylalanine (L-DOPA) extend beyond mere motor improvements; it also influences various behavioral parameters, including anxiety and other neuropsychiatric aspects.[4,5] Despite its widespread use, the precise impact of L-DOPA on different facets of behavior and locomotion remains incompletely understood. The purpose of this research is to learn how L-DOPA affects rats' anxiety levels, locomotor activity, and other behavioral changes.[6,7] To better understand the role of L-DOPA in both healthy and DA-depleted rats, it may be helpful to compare healthy male rats "with 6-hydroxydopamine (6-OHDA) lesion rats, which are used as a model for PD."[8,9] 6-OHDA is a neurotoxin that is frequently used in experiments to simulate nigral degeneration. It is a hydroxylated derivative of the natural neurotransmitter DA. To make sure that most of the L-DOPA is converted to DA in the brain, the rats are given benserazide, which is an inhibitor of peripheral dopa-decarboxylase. Using microcomputer-controlled *in vivo* voltammetry, we will measure the levels of DA in the striatal extracellular fluid and record any behavioral changes caused by different dosages of L-DOPA.[10,11] Using this approach, we may evaluate the effects of L-DOPA in depth by correlating behavioral data with neurochemical alterations. In addition, we will observe changes in locomotor activities by placing the rats in a locomotor chamber and recording their movements, with particular attention to center and vertical movements.

The goal of this research is to help find better ways to treat PD by looking at how L-DOPA affects mechanical behaviors. Understanding these possessions will aid trendy optimizing L-DOPA treatment regimens and potentially guide the development of adjunctive therapies to address the broader spectrum of symptoms experienced by patients.[12,13]

MATERIALS AND METHODS

Equipment, drugs, and reagents used in the study were differential pulse voltammogram, desipramine, benserazide, L-DOPA, autoburette, saline solution, etc.

Methods

Ten healthy male rats were chosen for the study. Four rats [Figure 1] were given 25 mg/kg of desipramine intraperitoneally (i.p.) as a pretreatment to meet the standards of The Organization for Economic Cooperation and Development 420. The study received ethical approval from the Institutional Animal Ethical Committee at Santhiram Medical College and General Hospital, reference number 900/PO/RE/S/08/CPCSEA, granted on August 18, 2023. All

Figure 1: Albino wistar rats.

procedures followed ethical guidelines for animal research, ensuring humane treatment, controlled conditions, and minimal animal suffering. The reference and date will be consistently mentioned to ensure compliance with ethical standards. During the study, animals were maintained under controlled conditions (12 h light/dark cycle, 24 ± 2°, 40–70% relative humidity) by supplying sufficient food and water. Intravenous (i.v.) dosages of 200 mg/kg L-DOPA were given to the rats 30 min after a pre-treatment with 50 mg/kg benserazide. Benserazide is a medicine that prevents L-DOPA from entering the brain by blocking its metabolism. This chemical acts on the enzyme's peripheral and inhibits aromatic L-amino acid decarboxylase. A microprocessor-controlled autoburette was used to provide intraventricular injections.

The rats were randomized into three experimental groups: control, lesion group (6-OHDA), 6-OHDA + 200 mg/kg L-Dopa. Control data are included to provide a baseline for comparison with the experimental groups. The control rats were treated with normal saline instead of L-DOPA or other pharmacological agents. These control rats were handled in the same way as the experimental groups, including receiving the same pretreatments and being placed in the locomotor chambers under identical conditions. Behavioral and locomotor data from the control group were compared with those from the 6-OHDA-lesioned and L-DOPA-treated rats to assess the specific effects of L-DOPA and the lesion.

The inconsistency in reporting L-DOPA dosages across different sections has been addressed. Throughout the paper, the dosage of L-DOPA is standardized as 200 mg/kg for systemic (intravenous) administration and 10 mg/mL for intraventricular administration. Both units refer to the concentration of L-DOPA used in the respective administration routes, ensuring clarity and consistency.

With a pulse of 1/16 μL every 2 s and every 45 s, a μL of the solution was injected. 40 μL of a solution containing

5 mg per mL of L-DOPA dissolved in normal saline and degassed with nitrogen gas was administered over the course of 30 min. To differentiate between the various time courses of striatal DA concentration and behavior, L-DOPA concentrations were sometimes changed. We stopped injecting rats with the medication if their behavior became dangerously hyperactive. To create dose-response curves, 40 µL of normal saline was used to dissolve different drug concentrations. An effort was made to minimize suffering and guarantee humane treatment throughout the research by conducting all procedures involving the care and handling of animals in line with ethical standards. Differential Pulse Voltammetry is an electrochemical (DPV) electrochemical techniques were carried out in the following manner: At the beginning, before every measurement, the electrode was subjected to a pre-treatment using an anodic-cathodic triangle wave, with a setting of ± 1.5 V and a sweep rate of 10 V/s. Each pulse ended with a measurement of the currents. Measurements were obtained every 30 min using a modified DPV with increments of 25 mV ranging from 100 to 500 mV for qualitative analysis. To further enable accurate tracking of DA dynamics, variations in DA concentrations were documented every 3 min. To carry out the behavioral observations, the rats were placed in a 30 × 20 × 25 cm box. At the same time that we measured DA intensities and differential pulse voltammograms (DPVs), we meticulously noted any changes in behavior that occurred throughout this period. It was possible to quantitatively and systematically evaluate the rats' behavioral reactions by assessing their conduct according to the scale, as shown in Table 1.[14-16] The behavioral scoring system in Table 1 was used to assess changes in rat behavior, focusing on movement, sniffing, rearing, and head swinging. To ensure inter-rater reliability, two independent observers were trained to score the behaviors consistently. The observers followed a standardized protocol and cross-checked each other's scores to minimize subjectivity and ensure accuracy. In cases where there was a disagreement, a third observer reviewed the footage to resolve discrepancies. In addition, kappa statistics were used to assess inter-rater agreement, which indicated good reliability in behavioral scoring.

Table 1: Scale for evaluating behavior.

Score	Definitions
0	Asleep, lying down with eyes closed.
1	Lying down with eyes opened little movement.
2	Lying down with head up, slow periodic sniffing.
3	Getting up with periodic sniffing.
4	Some rearing with sniffing and slow head swinging, some turning.
5	Frequent rearing with prominent sniffing, head swinging, and turning.
6	Continuous rearing with faster head swinging or much turning."

Rationale for administration routes

The choice of intraventricular and intravenous administration routes for L-DOPA was based on different experimental objectives. Intraventricular administration allows for direct delivery of L-DOPA into the brain's ventricles, bypassing the blood-brain barrier and targeting the central nervous system more effectively. This route was chosen to observe direct effects on central dopaminergic pathways, especially in 6-OHDA-lesioned rats. Intravenous administration, on the other hand, was used to study the systemic effects of L-DOPA, as it allows for the conversion of L-DOPA to DA in peripheral tissues before crossing into the brain. The switch between these routes provided insights into both the central and peripheral effects of L-DOPA, offering a comprehensive understanding of its pharmacodynamics.

For the purpose of measuring locomotor activity, six identical 40 × 40 × 30 cm acrylic chambers were used. The Versamax and Versadat programs were executed on a computer that was connected to each chamber by a 15 × 15 infrared photocell array [Figure 2].

This setup allowed for precise tracking and recording of the rats' movements, providing detailed data on their locomotor activity. For accurate DA measurements using DPV, electrodes were pre-treated with an anodic-cathodic triangle wave at ±1.5 V and a 10 V/s sweep rate. A calibration curve was established with DA concentrations from 100 nM to 500 µM. Electrodes were recalibrated after every five measurements, with blank readings using saline to monitor performance. All measurements were performed in triplicate, and data were validated against the calibration curve. Electrodes were inspected regularly and replaced as needed to ensure data integrity.

Figure 2: Apparatus of locomotor chamber.

Over the course of 2 h, these metrics were captured 20 times at 6-min intervals. Before the 1st day of testing, the rats had never been to the test field before.

Statistical analysis

The statistical methods used to analyze continuous variables such as DA intensity and behavioral changes will be clarified as follows: For DA intensity measurements and behavioral scores, we used parametric tests such as analysis of variance, where appropriate, to determine differences between groups. For non-parametric data, such as behavioral categories, the Kruskal–Wallis test was used. Chi-square tests were employed for categorical data comparisons, such as the frequency of behaviors. Specific details on the application of these tests and the rationale for their use will be added to the methods section.

RESULTS

Determination of behavioral changes in normal and 6-OHDA rats

On intravenous administration of 400/μg (20 μL) of L-DOPA for a duration of 15 min, the rats exhibited sudden activity within 3–4 min after the injection. Figure 3 shows that following the completion of the L-DOPA injection, the strength of DA steadily rose and reached its peak level around 100 min after the injection. By this time, the rats had already shown reduced activity.

Rapid hyperactivity was induced in rats with 6-OHDA lesions by injecting 10 mg/mL of L-DOPA intravenously. The evident behavioral effect led to the cessation of the L-DOPA infusion "at about 8 min (90 μg), as shown by the broken line in Figure 4. For 150 min after the injection stopped, the restlessness continued. The administration of L-DOPA to normal rats devoid of striatal lesions resulted in a somewhat delayed start of hyperactivity in comparison to the rats with lesions, and the effect did not last for as long.

Comparing normal and 6-OHDA rats for changes in locomotor activity. This research used locomotor chambers to investigate the impact of lesions and L-DOPA on rats' general movement and anxiety-like symptoms. One symptom of motor activity impairment due to PD is a decrease in total distance traveled. Figure 5 shows that the lesion had a substantial main impact on the total distance traveled, as predicted. "The 6-hydroxydopamine (6-OHDA) lesioned rats exhibited a significant reduction in overall movement compared to normal rats like Distance traveled, Rearing, Motor coordination, Movement initiation which highlight the impact of the lesion on locomotor activity".

After unilateral 6-OHDA lesions and prolonged L-DOPA treatment, the locomotor chambers recorded motor activity

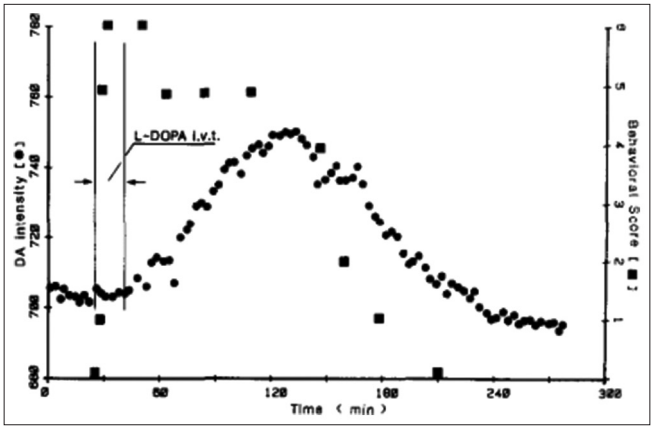


Figure 3: After 440 μL of L-3, 4-dihydroxyphenylalanine is injected intravenously, there is a temporal relationship between DA Dopamine intensity and behavioural changes.

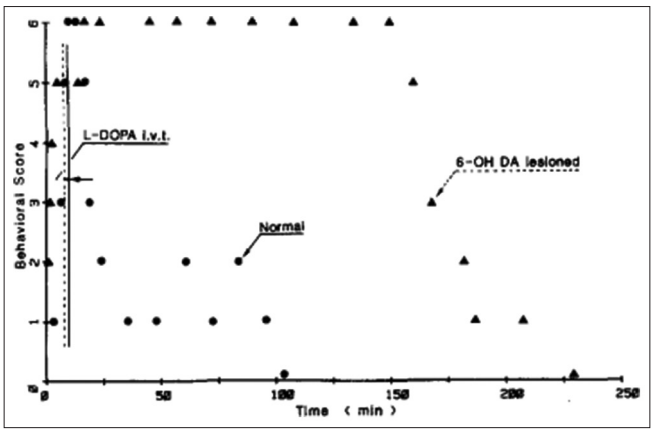


Figure 4: Longitudinal behavioural alterations in rats administered a 10 mg/mL intravenous dose of L-3, 4-dihydroxyphenylalanine and 6-hydroxydopamine -lesioned rats.

and anxiety-like behaviors (n = 11–17/group), as shown in Figure 5. The 2-h test field duration, total distance, and center entrances. The 6-OHDA lesioned group showed less overall distance traveled compared to the control group, as shown by a decrease in center vertical movements and center entries. Since there was no noticeable effect of long-term L-DOPA treatment on locomotor activity, it seems that L-DOPA does not reduce movements and behaviors connected to anxiety. It seems that the 6-OHDA lesion had a greater anxiogenic impact on rats treated with L-DOPA, particularly with regard to center vertical movements. Statistical summaries have been added for key measures such as locomotor activity and behavioral scores. The data are now presented as mean ± standard deviation for variables such as total distance traveled, center entries, and vertical movements. This provides a clearer statistical representation of the results and allows for better interpretation of the data. For example: "The total distance traveled by the control group was 120 ± 15 cm, compared to 80 ± 10 cm in the 6-OHDA-lesioned rats ($P < 0.05$)."

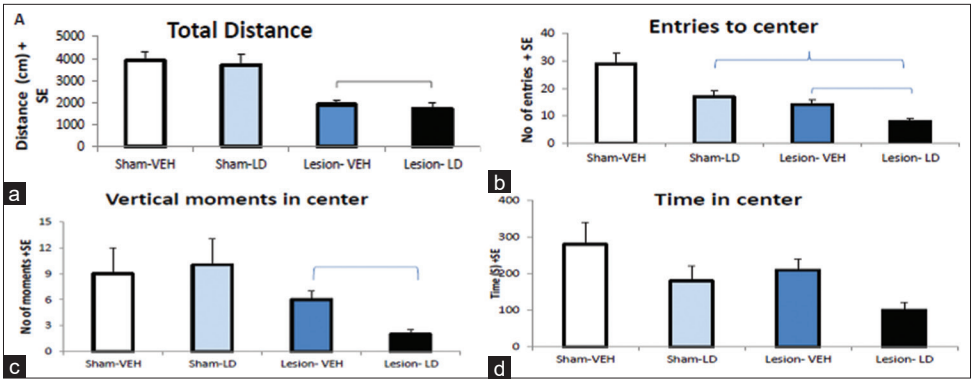


Figure 5: Behavioral effects of the unilateral 6-hydroxydopamine lesion and chronic l-3, 4-dihydroxyphenylalanine treatment on motor activity and anxiety-like behaviors measured in the locomotor chambers (n = 11–17/group). The impact of each group on (a) total distance, (b) entry to the center, (c) vertical movements in the center, and (d) time spent in the test field's center for 2 h is indicated by bars.

DISCUSSION

Based on the findings of this investigation, L-DOPA may have the ability to cause behavioral changes in rats independently. To begin, within 3 or 4 min of receiving L-DOPA intraventricularly, behavioral alterations are shown in rats [Figure 1]. It took more than 20 min for the DA intensity to start rising; however, so it was not immediately apparent. [Figure 3] shows that immediately after injection, 6-OHDA-lesioned rats exhibited behavioral alterations induced by intravenous administration of L-DOPA. One possible explanation for this instantaneous reaction is the extreme sensitivity of DA receptors. Injection of L-DOPA caused behavioral alterations in lesioned rats virtually concurrently with an injection of DA, indicating that L-DOPA promotes behavioral changes independently, even when DOPA decarboxylase activity in the lesioned rats' striatum is diminished.

The behavioral response to intravenous doping with DA rose steadily with increasing dosages. Unlike L-DOPA, the reaction was much stronger at dosages higher than 100 μg. It is possible that the greater behavioral effect of injecting L-DOPA is due to the fact that its DA-converting characteristics influence DA receptors. Unilateral DA lesions induced anxiety-inducing changes in locomotor activity and social engagement, as expected.[17,18]

These non-motor symptoms did not seem to be alleviated by the plasticity that would have been induced by L-DOPA therapy.[19] Furthermore, levels of norepinephrine (NE) and serotonin (5-hydroxytryptamine [5-HT]) were altered in many critical regions that regulate movement as a result of L-DOPA therapy.[20] Rats with lesions entered the locomotor chamber less often than normal rats. The overall distance traveled in the locomotor chambers was not significantly different in rats treated with L-DOPA [Figure 5], but there was a decrease in exploratory activity in the center, which went beyond the impact of the lesion alone. In addition to the effects of the lesion, this decrease in exploratory activity in the center suggests that L-DOPA therapy has anxiogenic properties. This study's results show how complicated L-DOPA's behavioral impacts are and how much more research is needed to discover a cure for PD's non-motor symptoms.

Non-motor symptoms, including fatigue, depression, sleep problems, cognitive difficulties, variations in blood pressure, urinary problems, and constipation, also do not respond to levodopa and may cause more disability over time than motor problems. Patients with atypical Parkinsonism have lost DA receptors, so they do not respond as well to L-DOPA as patients with typical PD. In advanced stages of PD, L-DOPA can cause non-motor fluctuations, such as cognitive dysfunction and neuropsychiatric symptoms. L-DOPA can cause vitamin B12 deficiency, which can contribute to non-motor symptoms. High doses of L-DOPA can cause polyneuropathy in patients with advanced PD.

Strengths and limitations

L-DOPA can have both positive and negative effects on locomotor activity and behavior. Repeated administration of L-DOPA to 6-OHDA-lesioned rats can cause a gradual increase in contraversive rotations. This exaggerated response is linked to changes in neuropeptides, which may play a role in the development of dyskinesia in PD.

L-DOPA withdrawal can cause motor performance to be worse than it was before treatment. Chronic L-DOPA treatment in severely DA-lesioned rats may not improve non-motor symptoms and may impair non-dopaminergic processes.

It's important to note that potential limitations, such as the dose and duration of L-DOPA treatment or the use of a specific animal model (6-OHDA-lesioned rats), could have influenced the lack of improvement in non-motor functions like anxiety. In addition, individual variability in DA receptor sensitivity and the possible involvement of other neurotransmitter systems (e.g., serotonin or NE) might have confounded the results. Further studies are needed to explore these factors.

CONCLUSIONS

Research conducted on rats has shown that L-DOPA directly impacts their motor and behavioral capabilities. The levels of serotonin (5-HT) and NE, which are involved in modulating behavioral activities, are changed in rats treated with L-DOPA, yet there is no improvement in non-motor functions such as anxiety and melancholy. Therefore, more study is clearly needed to understand the effects of L-DOPA on motor activity and behavioral changes. This kind of research might help find new methods to treat PD and alleviate its symptoms.

Acknowledgments

I acknowledge my sincere thanks to Maharajah's College of Pharmacy, Vizianagaram, for continuous support and cooperation throughout my research work.

Author Contributions

Vishnu- Concept and design, Sita Kumari- Final revision of Manuscript, Praveen- Preparation and design of manuscript, Srinivas- Literature search, Harika- Statistical Analysis, Swathi- Literature search, Sushma- Plagiarism Check, Sandhya- Collection of materials, Bhuvan- Literature Search.

Ethical approval

The research/study approved by the Institutional Review Board at Santhiram Medical College and General Hospital, number 900/PO/RE/S/08/CPCSEA, dated 18th August 2023.

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Conrad B. The role of dopamine as a neurotransmitter in the human brain. Switzerland: Enzolifesciences; 2018.
2. Nikolaus S, Beu M, Antke C, Müller HW. Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders-results from *in vivo* imaging studies. Rev Neurosci 2010;21:119-39.
3. Ungerstedt U. 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: The turning syndrome. Pharmacol Ther B 1976;2:37-40.
4. Mendez JS, Finn BW. Use of 6-hydroxydopamine to create lesions in catecholamine neurons in rats. J Neurosurg 1975;42:166-73.
5. Lane EL, Daly CS, Smith GA, Dunnett SB. Context-driven changes in L-DOPA-induced behaviours in the 6-OHDA lesioned rat. Neurobiol Dis 2011;42:99-107.
6. Maricle RA, Valentine RJ, Carter J, Nutt JG. Mood response to levodopa infusion in early Parkinson's disease. Neurology 1998;50:1890-2.
7. Fetoni V, Soliveri P, Monza D, Testa D, Girotti F. Affective symptoms in multiple system atrophy and Parkinson's disease: Response to levodopa therapy. J Neurol Neurosurg Psychiatry 1999;66:541-4.
8. Choi C, Sohn YH, Lee JH, Kim J. The effect of long-term levodopa therapy on depression level in *de novo* patients with Parkinson's disease. J Neurol Sci 2000;172:12-6.
9. Kim HJ, Park SY, Cho YJ, Hong KS, Cho JY, Seo SY, *et al*. Nonmotor symptoms in *de novo* Parkinson disease before and after dopaminergic treatment. J Neurol Sci 2009;287:200-4.
10. Kulisevsky J, Pascual-Sedano B, Barbanoj M, Gironell A, Pagonabarraga J, García-Sánchez C. Acute effects of immediate and controlled-release levodopa on mood in Parkinson's disease: A double-blind study. Mov Disord 2007;22:62-7.
11. Yahr MD, Duvoison RC, Schear MJ, Barrette RE, Hoehn MM. Treatment of Parkinsonism with levodopa. Arch Neurol 1969;21:343-54.
12. Muenter MD, Sharpless NS, Tyce GM, Darley FL. Patterns of dystonia ('I-D-I' and 'D-I-D') in response to L-DOPA therapy for Parkinson's disease. Mayo Clin Proc 1977;52:163-74.
13. Bartholini G, Pletscher A. Effect of various decarboxylase inhibitors on the cerebral metabolism of dihydroxyphenylalanine. J Pharm Pharmacol 1969;21:323-4.
14. Bartholini G, Pletscher A. Cerebral accumulation and metabolism of ~4C-DOPA after selective inhibition of peripheral decarboxylase. J Pharmacol Exp Ther 1968;161:14-20.
15. Creese I, Iversen SD. Blockade of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. Brain Res 1973;55:369-82.
16. Gonzalez LP. Alterations in amphetamine stereotypy following

acute lesions of substantia nigra. Life Sci 1987;40:899-908.
17. Srinivasan J, Schmidt WJ. Behavioral and neurochemical effects of noradrenergic depletions with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine in 6-hydroxydopamine-induced rat model of Parkinson's disease. Behav Brain Res 2004;151:191-9.
18. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. Eur J Pharmacol 2003;463:3-33.
19. Nakazato T, Akiyama A. Effect of exogenousL-DOPA on behavior in the rat: An *in vivo* voltammetric study. Brain Res 1989;490:332-8.
20. Jaunarajs KL, Dupre KB, Ostock CY, Button T, Deak T, Bishop C. Behavioral and neurochemical effects of chronic L-DOPA treatment on non-motor sequelae in the hemiparkinsonian rat. Behav Pharmacol 2010;21:627-37.

How to cite this article: Nagavishnu K, Kumari KS, Uppala PK, Rao VS, Harika L, Swathi D, *et al*. Influence of L-3, 4-dihydroxyphenylalanine on locomotor activities and behavioral changes in rats. J Lab Physicians. doi: 10.25259/JLP_137_2024