EVALUATION OF ODAP DEPENDENT DOWN REGULATION OF RKIP AS A RISK FACTOR IN PROGRESSION OF PARKINSON'S DISEASE IN EXPERIMENTAL ANIMALS

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Abstract:
β-N-Oxalyl-L-α, β-diaminopropionic Acid (L-ODAP) a neurotoxin and glutamate analogue from *Lathyrus sativus* seeds known to cause Neurolathyrism. Recent studies from our laboratory, indicated that L-ODAP induces Hypoxia and down regulates PEBP1. Hippocampal cholinergic neuro stimulating peptide (HCNP), increases acetylcholine synthesis derivative of HCNP precursor protein (HCNPpp), known as PEBP1 Decreased expression of PEBP correlated with accumulation of Abeta. Several publications indicated that down-regulation of PEBP results in the progression of Parkinson's Disease (PD). We hypothesize that consumption of Lathyrus pulse might be a risk factor for PD. Thus, the present proposal aims to explore chemically induced PD mice models and control mice fed with the Lathyrus diet and score for PD. we investigated behavioral parameters like tremors, rigidity, akinesia, and postural reflexes by inducing Parkinsonism in experimental animals (C57BL/6 strain mice n=6) using the injection of neurotoxins MPTP. We analyzed the data of statistical and histopathological evidence that L-ODAP does not cause any neurological damage to induce Parkinsonism in mice. The current study supports fresh statements that *L. sativus* is a hypothetically important useful food with high value to human health in areas of cardiovascular physiology, hypoxia, and nutrition since it contains both homoarginine and L-ODAP. According to a scientific study, consuming *L. sativus* as part of a regular diet is beneficial to health.

Keywords: β-N-Oxalyl-L-α, β-diaminopropionic Acid, Hippocampal cholinergic neuro stimulating peptide (HCNP), PEBP1, Parkinson Disease, Lathyrus sativus.

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INTRODUCTION:

β-N-Oxalyl-L-α,β-diaminopropionic Acid (L-ODAP) a neurotoxin and glutamate analog from *Lathyrus sativus* seeds known to cause neurolathyrism a disease associated with upper motor neuron damage and paralysis. It is also known as a mitochondrial toxin. Recent studies from our laboratory[1] indicated that L-ODAP induces hypoxia and down-regulated PEBP1-phosphatidylethanolamine binding protein. Hippocampal cholinergic neuro stimulating peptide (HCNP), which enhances acetylcholine synthesis is derived from HCNP precursor protein (HCNPpp), known as PEBP1. Decreased expression of PEBP correlated with accumulation of Abeta. Several publications indicated that down-regulation of PEBP results in the progression of Parkinson's Disease (PD). Apart from neurodegeneration of the nigrostriatal dopaminergic pathway, disturbances of the serotonergic, noradrenergic, glutamatergic, GABAergic, and cholinergic systems were also affected from the angle of the pathophysiology of PD[2, 3].

We hypothesize that consumption of Lathyrus pulse might be a risk factor for PD. Down-regulation of PEBP may result in lower levels of HCNP that may contribute to neuronal dysfunction and pathogenesis. Thus, the present proposal aims to explore chemically inducedPD mice models and control mice fed with the Lathyrus diet and score for PD.

MATERIALS AND METHODS:

Animals: The animals were divided into four groups, each group consisting of six C57BL/6 strain mice (n=6). The following drugs were administered to mice by intraperitoneal route.

Group-1 (Control): Normal Saline.

Group-2 (Standard): 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Group-3: *β*-N-oxalyl-L-*α,β*-diaminopropionic acid (L-ODAP).

Group-4: 50% MPTP + 50% *β*-N-oxalyl-L-*α,β*-diaminopropionic acid (L-ODAP).

Parkinsonism was induced in experimental animals by the injection of neurotoxins MPTP. The behavioral parameters like tremors, rigidity, akinesia and postural reflexes were recorded[4].

Muscular coordination test- Rotarod: The test process involves the choice of mice based on their withstanding ability of 120 seconds on the rotating rod. Each mouse from the four groups was individually placed on the rotating rod and the fall-off time (Sec) of mice from the rotating rod was recorded[5, 6]. Muscle grip strength is an important tool to show the muscle coordination ability of animals which is mainly impaired in Parkinson's disease due to loss of dopamine. To evaluate the muscle coordination ability of the animal Rota-rod apparatus was used and the fall-off time was recorded. Histopathological studies were also conducted to correlate the results. The results are given in mean ± SD, a one-way ANOVA test was conducted using IBM-SPSS software, also the least square difference method was applied to identify the difference between the groups statistically.

Locomotor activity- Actophotometer: The locomotor activity works on photoelectric cells. This is connected in a circuit with a counter which can be easily measured on actophotometer. The units of the activity counts were based on the beam breaks by the movement of the mice. The spontaneous locomotor activity of each mouse was recorded individually for 10 minutes. At the end of the study period, the animals were sacrificed to study brain histopathology[5].

RESULTS AND DISCUSSION:

Behavioral parameters: Behavioral parameters like tremors, rigidity, akinesia and postural reflexes were absent in the control group and L-ODAP groups. Tremors, rigidity, and akinesia were mild; postural reflexes were moderate in group-4 animals. All the behavioral parameters were severe in MPTP-administered animals[7].

Table 1: Behavioral parameters

Characteristic feature	Group-1	Group-2	Group-3	Group-4
	Control	MPTP	L-ODAP	(50%)*(L-ODAP+MPTP)
Tremors	-	+++	-	+
Rigidity	-	+++	-	+
Akinesia	-	+++	-	+
Postural reflexes	-	+++	-	++

-Absent, +Mild, ++Moderate, +++Severe

Rotarod test: The muscle grip strength was evaluated on the rotating rod. The results show that the control group mice fall-off from rotarod as 129.33 ± 3.67. The brain histopathology shows normal structural features in this group of animals (Fig. 3).

The animals treated with MPTP show the muscle grip as 47.17 ± 2.86. The activity was reduced statistically when compared with control group animals ($p<0.5$). The histopathology also confirms the same with multi-focal necrosis with infiltration of inflammatory cells and hemorrhages in the cerebral cortex region of the brain (Fig. 4)[8].

The group-3 treated with L-ODAP shows the muscle grip as 128.17 ± 3.49. The results say that there was no significant difference between the control group and the animals treated with L-ODAP at $p<0.05$. Even the histopathological studies also showed normal morphological structures in the brain (Fig. 5).

The group-4, treated with (50%)*(MPTP+ L-ODAP) was 86.33 ± 3.67. The results say that there was a statistical difference between the MPTP-administered group and group-4 ($p<0.05$). The histopathological studies also confirm the same with Mild infiltration of inflammatory cells in meninges and thickening of meninges covering the cerebral cortex, which may be dependent on the dose of MPTP (Fig. 6)[9].

Fig. 1: Study of muscle grip strength by Rota-rod apparatus

Table 2: Muscle grip strength of mice by rota-rod apparatus

	Group-1	Group-2	Group-3	Group-4
MICE NUMBER	**Control**	**MPTP**	**L-ODAP**	**(50%)*(L-ODAP+MPTP)**
1	128	43	131	89
2	125	48	129	84
3	126	51	133	88
4	134	47	125	85
5	130	45	124	81
6	133	49	127	91
MEAN	**129.33***	**47.17**	**128.17***	**86.33***
STANDARD DEVIATION	3.67	2.86	3.49	3.67

*$p<0.05$, compared with the MPTP-administered group.

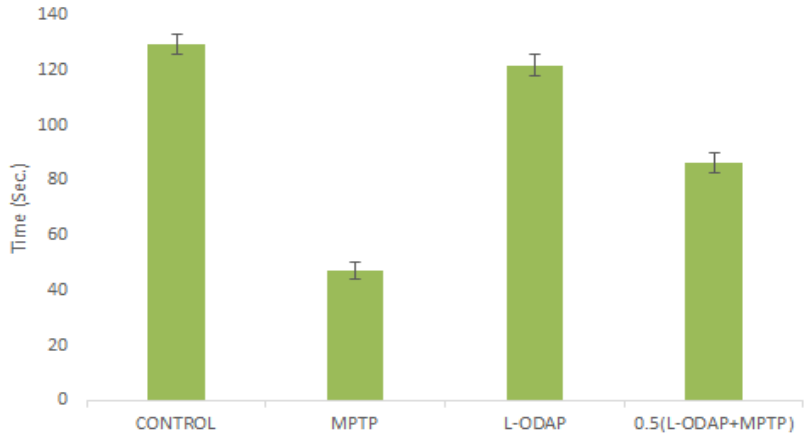

Fig. 2: Muscle grip strength by rota-rod apparatus

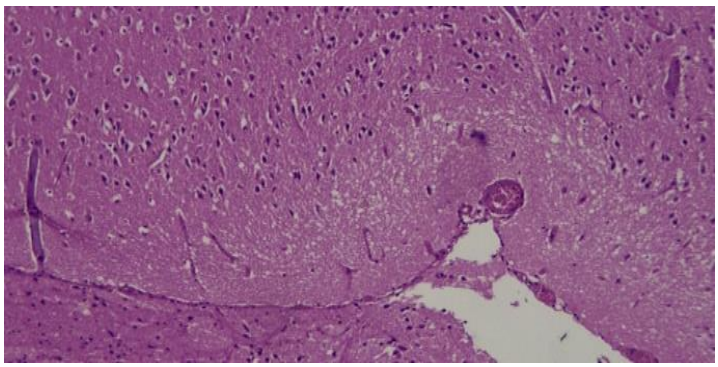

Fig. 3: Normal morphology of frontal/ cerebral cortex of brain

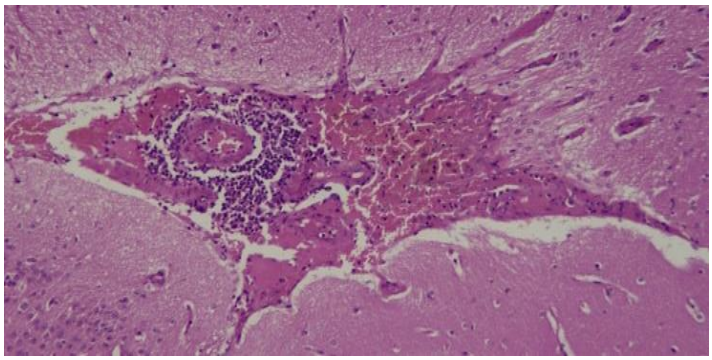

Fig. 4: Multi-focal necrosis with infiltration of inflammatory cells and haemorrhages in the cortex region of the brain

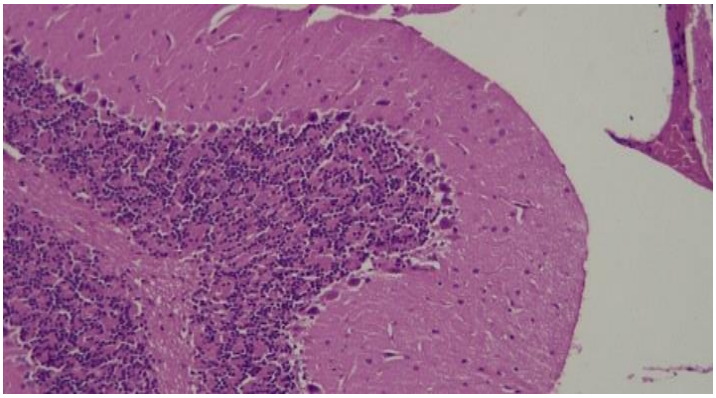

Fig. 5: Normal morphology of cerebellum

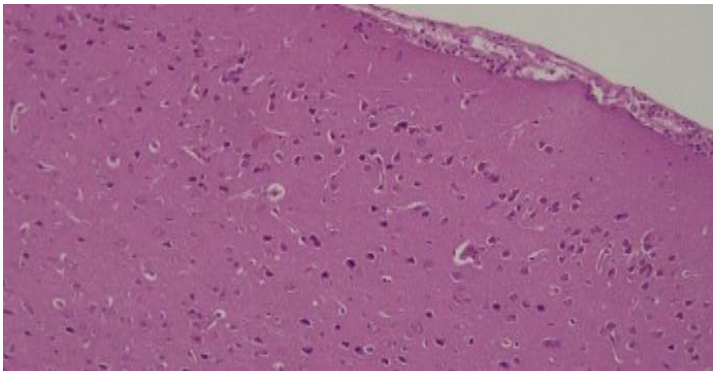

Fig. 6: Mild infiltration of inflammatory cells in meninges and thickening of meninges covering the cerebral cortex

Actophotometer test: Locomotor activity was determined on all four group mice by placing the individual mice in an activity cage and the number of beams cut off by mice was recorded. The results are given in table 2.

Fig. 7: Study of locomotor activity

Table 3: Locomotor activity of mice

	Group-1	Group-2	Group-3	Group-4
MICE NUMBER	**Control**	**MPTP**	**L-ODAP**	**(50%)*(L-ODAP+MPTP)**
1	82	40	79	81
2	80	41	76	79
3	78	37	75	74
4	83	45	84	78
5	81	43	78	72
6	85	38	81	71
MEAN	**81.50***	**40.67**	**78.83***	**75.83***
STANDARD DEVIATION (SD)	**2.43**	**3.01**	**3.31**	**4.07**

*p<0.05, compared with the MPTP-administered group.

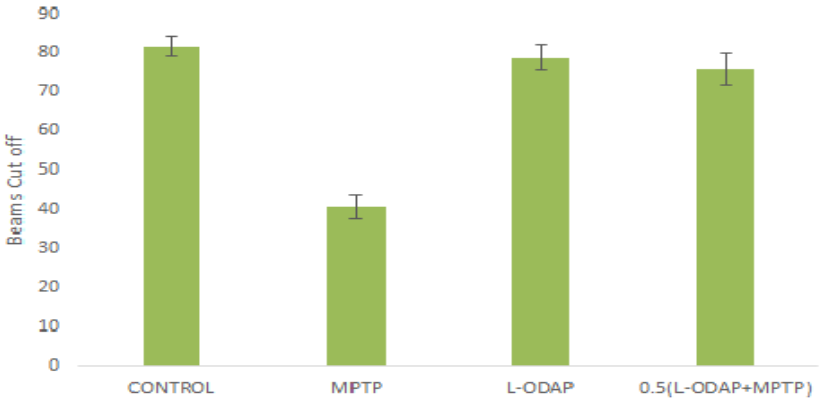

Fig. 8: Beam Cut off by animals in actophotometer

Group-1 (Control) shows the locomotor index as 81.50 ± 2.43. The histopathological report shows normal morphology (Fig. 9).

The locomotor index in group-2, animals treated with MPTP was 40.67 ± 3.01. The results say that, the statistical difference between control (group-1) and MPTP groups at p<0.05. The histopathological study also confirms that pathological changes like multi-focal apoptosis/ necrosis of neuronal cells in the hippocampus of the brain were observed in the brain of the mice with MPTP administration (Fig. 10).

The locomotor index in group-3, animals treated with L-ODAP was 78.83 ± 3.31. The results say that there was no statistical difference between the MPTP-treated group (p<0.05). Even the histopathological studies also confirm the same with normal morphology reports (Fig. 11).

The locomotor index in group-4, treated with (50%)*(MPTP+ L-ODAP) was 75.83 ± 4.07. The results say that there was a statistical difference between the MPTP-treated group and group-4 (p<0.05). The histopathological studies also confirm the same with moderate changes like foci of infiltration of inflammatory cells in the cerebral cortex of the brain in the brain (Fig. 12).

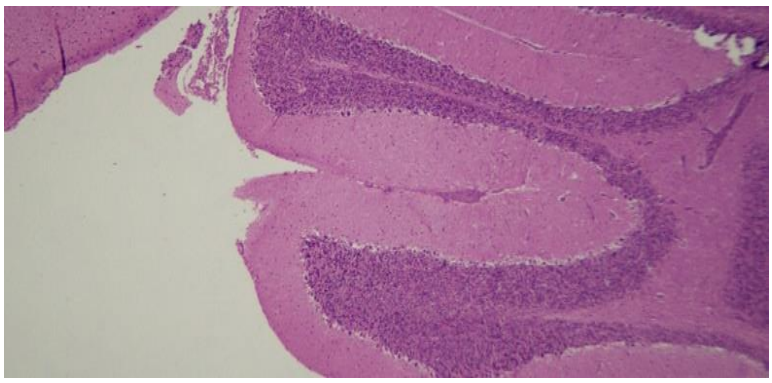

Fig. 9: Normal morphology of cerebellum

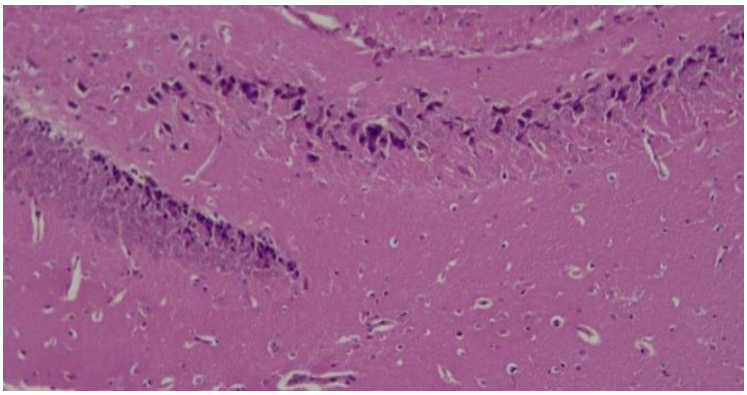

Fig. 10: Multi-focal apoptosis/ necrosis of neuronal cells in the hippocampus of the brain

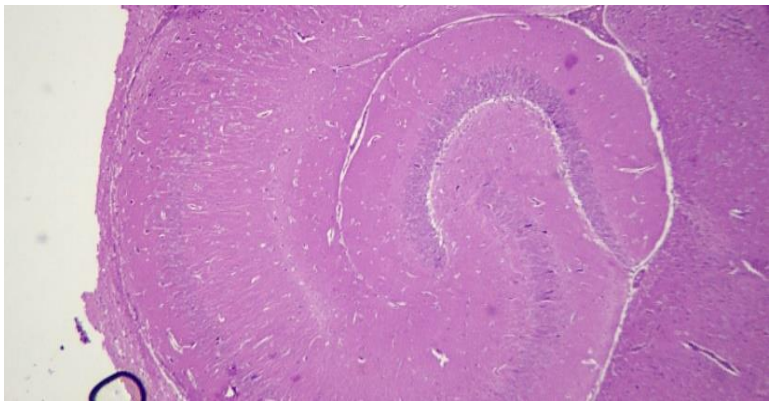

Fig. 11: Normal morphology of hippocampus of the brain

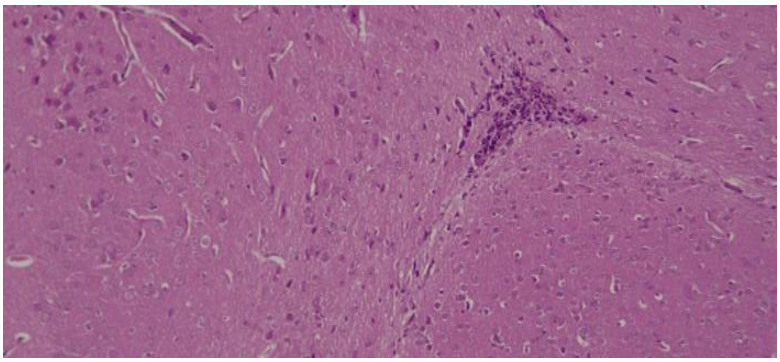

Fig. 12: Foci of infiltration of inflammatory cells in the cerebral cortex of the brain

CONCLUSION:

There was no statistical and histopathological evidence that *β*-N-oxalyl-L-*α,β*-diaminopropionic acid (L-ODAP) causes neurological damage to induce Parkinsonism in mice. In fact, it helped relieve the ill effects of MPTP in these mice although the effect was statistically significant at the tested dose and frequency. The present study

supports recent claims that *L. sativus* is a potentially significant functional food with high value to human health in areas of cardiovascular physiology, hypoxia, and nutrition since it contains both homoarginine and L-ODAP. According to a scientific study, consuming *L. sativus* as part of a regular diet is beneficial to health[6]. For use as a hemostatic agent, L-ODAP has already been granted a Chinese patent[10], and more recently a. US patent[11].

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