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Beneficial effects of long term caloric vestibular stimulation on changes in brain neurotransmitter dopamine and locomotor activity in Parkinson's Disease induced rats

Thanalakshmi J[1], Archana R[*1], Senthil Kumar S[2]

[1]Department of Physiology, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 602105, Tamilnadu, India
[2]Department of Research and Development, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 602105, Tamilnadu, India

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ABSTRACT

Parkinson's Disease (PD) is a neurodegenerative disorder caused due to deficiency of Dopamine in the Substantia nigra. The existing pharmaceutical treatments are not meeting the need, whereas deep brain stimulation is not suitable for patients with co-morbidities. Therefore, a need for non-invasive and conventional treatment with fewer side effects is required. So we have tried a simple method of Caloric Vestibular Stimulation (CVS) for the long term and assessed its neuroprotective effect in PD induced rats. In the present study, 30 adult male Wistar albino rats (250 - 300g) were randomly assigned into five groups. Group 1 control, Group 2 was induced with Parkinson's disease using rotenone, Group 3 was PD induced and CVS gave for 45 days, Group 4 was PD induced for 21 days and left untreated to study for its recovery, Group 5 was given CVS only for 45 days. The behavioral activity was recorded using an actophotometer and to assess the function of the nigrostriatal pathway. Dopamine produced in the striatum was measured using reverse-phase HPLC. Results showed significant ($P< 0.05$) alteration in dopamine and locomotor activity in PD, which was significantly ($P< 0.05$) improved by warm water CVS administration in Parkinson's disease-induced rat. In this aspect, CVS can be utilized as a conventional treatment method for PD and thus recommended for further investigations towards translational treatment in humans for Parkinson's disease.

*Corresponding Author

Name: Archana R
Phone: +91-9840608149
Email: dr.rarchana@gmail.com

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INTRODUCTION

Parkinson's Disease (PD) is a neurodegenerative disorder caused by progressive degeneration of dopaminergic neurons in pars compacta of the substantia nigra, causing subsequent loss of axonal terminals in the corpus striatum. It is known to affect the nigrostriatal dopaminergic pathway in Parkinson's Disease (PD) (Fearnley and Lees, 1991). This results in motor incoordination and reduced motor activity associated with rigidity and tremor. Parkinson's Disease is associated with pathological features like loss of dopaminergic neurons and the presence of neuronal protein aggregates known as Alpha-synuclein, subsequently leading to reduced

dopamine secretions (Tieu, 2011). The molecular mechanism behind the genesis of PD is mitochondrial complex I inhibition and release of free radicals in circulation, leading to neuronal apoptosis (Narasimhan *et al.*, 2019). The dopamine-based medications given for a long duration will exceed the normal concentration of dopamine available in the midbrain (Yabe *et al.*, 2014). The alternate treatment methods like deep brain stimulations were not suitable for the older population as it fails to address motor incoordination (Sharma *et al.*, 2019). In this regard, new conventional treatment methods are required to overcome the neurochemical changes in the brain of Parkinson's disease. Caloric Vestibular Stimulations (CVS) are known to improve motor deficits in neurological disorders like Alzheimer's disease and Parkinson's Disease. (Suzuki *et al.*, 2001; Gopinath *et al.*, 2015). The CVS is a simple and non-invasive technique that triggers the vestibular nucleus through the afferent vestibular nerve. It subsequently leads to the activation of contralateral cortical structures (Suzuki *et al.*, 2001). Previous studiesobserved that stimulation of the vestibular apparatus increases acetylcholine release from rat hippocampus during anxiety and depression. It is also known to enhance memory by activating through a cholinergic pathway of the hippocampus (Horii *et al.*, 1994; Sailesh *et al.*, 2014b). However, the literature evidence for the beneficial effect of CVS on the prevention of Parkinson's Disease induced by rotenone is undetermined. For that reason, the present study was conducted to find the efficacy of bilateral warm water CVS in protecting dopaminergic neuronal loss in rats of Parkinson's Disease induced by intraperitoneal injection of Rotenone.

MATERIALS AND METHODS

Experimental design

Thirty Wistar male albino rats weighing around 250 - 300g were grouped as follows with each group containing 6 animals.

Grp 1: Control animal with no intervention

Grp 2: PD (administrated rotenone 3mg/kg body weight/day i.p mixed with vehicle; 98% Olive oil and 2% DMSO for 21 days)

Grp 3: PD+CVS: PD induced and CVS gave for 45 days

Grp 4: PD R (PD induced for 21 days and left untreated to study for its recovery)

Grp 5: CVS (Only intervention has given)

All animals were kept under environmentally controlled conditions with food and water adlibitum. The Parkinson's Disease was induced in the animal by intraperitoneal injection of Rotenone at a dose of 3mg/kg body weight for 21 days. The Rotenone was administered after mixing with 98%DMSO and 2% olive oil as a vehicle (Cannon *et al.*, 2009). The present work was approved by the Institutional Animal Ethical Committee of Saveetha Medical College and Hospital [IEC no: SU/CLAR/RD/006/2018].

In order to avoid change in the results due to variations in accordance with circadian rhythms of Dopamine level, all the experiments involving estimation of Dopamine was done between 8 am to 10 am. After the intervention of vestibular stimulation for 45 days following induction of Parkinson's Disease, the animals were sacrificed by cervical disruption and brain were removed quickly for HPLC estimation of Dopamine.

Caloric Vestibular Stimulation (CVS) procedure

The rat middle ear was irrigated with warm water of 42°C. Bilateral vestibular stimulation was done by irrigating each ear with 2.0ml of warm water administered at a flow rate of about 0.1 ml/sec using the syringe for 2 minutes (Sailesh *et al.*, 2014a).

Behavioral Parameter

Actophotometer was used to assess the locomotor activity of the animal, which operates based on the emission of photoelectric cells which are connected in circuit with a counter. Based on the movement of an animal inside the closed square arena the count was recorded for 180 seconds (Tieu, 2011)

Standard Preparation of dopamine

The primary standard of dopamine hydrochloride was prepared by dissolving 10 mg of compounds with 10 ml methanol in a volumetric flask (1 mg/ml). From the above standard stock solution, series of dilutions viz., 0.125 μg/ml, 0.500 μg/ml,0.750 μg/ml and 1 μg/ml were prepared in the methanol and was used as 100 % target concentration.

Sample preparation

The dopamine in the corpus striatum of the rat brain was estimated as done by Wagner *et al.* (1982) The rats were sacrificed by cervical dislocation. The brain was rapidly removed by placing on an ice-cold plate and corpus striatum was dissected out. The corpus striatum was homogenized with perchloric acid, followed by centrifugation. The homogenate was centrifuged in a refrigerated centrifuge (12000 rpm, 4°c) for 2 minutes and the supernatant was collected. The collected supernatant was again centrifuged at (12000 rpm, 4°c) for 20 minutes. The supernatant was filtered with a 0.22μm membrane

filter and 20μl of the sample was injected into the Rheodyne injector of the HPLC system.

Estimation of Dopamine (DA)

Determination of Dopamine hydrochloride (DA) was done using High-performance liquid chromatography with a PDA detector. The homogenate samples of rat striatum employing the direct UV range of the neurotransmitters are used. The method has been optimized and validated. The analytics were separated in 10 min on a reversed-phase column (C_{18}) with 0.5 % Acetic acid buffer & methanol (70:30) as the mobile phase; the flow rate was 1 ml/min. The UV measurements were carried out at 277 nm. The calibration curve for DA was linear up to about 1 g/ml, with a coefficient of determination (r^2) of 0.9995 with a lower limit of quantification of 0.118 g/ml. Since the procedure does not involve sample pre-purification or derivatisation, the recovery ranged from 97% to 102% and relative standard deviation (RSD) was better than 1.910 %; the use of the internal standard is not mandatory.

STATISTICAL ANALYSIS

All the experimental data were statistically analyzed by SPSS statistical software version 20. One way ANOVA followed by Tukey's HSD was done to compare the mean difference between the groups. P<0.05 was considered significant.

RESULTS AND DISCUSSION

The level of Dopamine expressed as mg/L in all the groups studied is summarized in (Figure 1). The results are expressed as mean ± SD. Parkinson's Disease group (Figure 1) showed a significant decrease (p<0.05) in dopamine concentration (0.18±0.03) when compared to the control group (0.46±0.09) in the corpus striatum. The intervention group, i.e., PD+CVS 45 days (0.24±0.03), showed significant improvement in (p<0.04) dopamine level when compared to the PD group. The PD R group has also shown a significant (0.20±0.03) decrease in the concentration of Dopamine, indicating that the rotenone has brought irreversible damage to the nigrostriatal neurons, whereas only CVS group (0.40±0.08) showed similar results as that of control.

The activity score results, as measured in the actophotometer on the 45 days, were found to be reduced in the PD group (83±27) when compared to the control group rats (302±26). Vestibular stimulation for 45 days (p < 0.04) has significantly increased the activity score (263±37), whereas the PD R, Figure 2 has shown a significant (p<0.03)

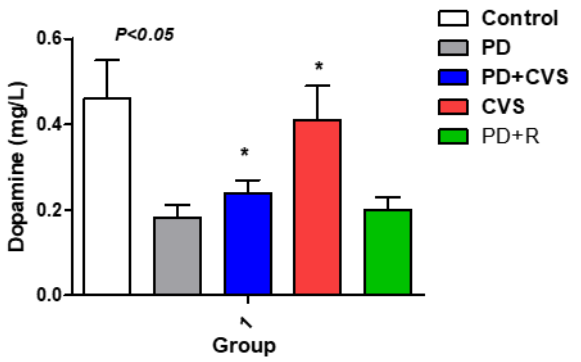

Figure 1: Dopamine concentration (mg/L) in the corpus striatum of control and study group

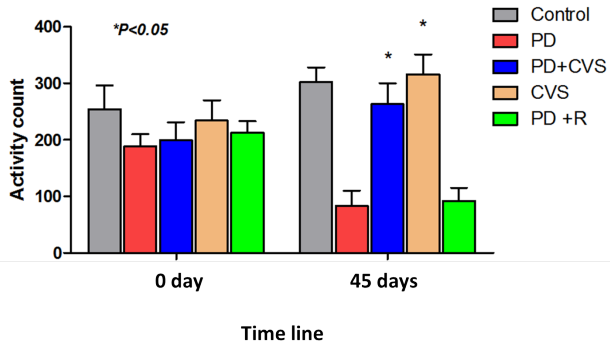

Figure 2: Activity score estimation by Actophotometer

decrease in activity score (92±23) (Figure 2).

The present study showed that CVS for the period of 45 days improves the dopamine level in the corpus striatum and motor activity in rotenone-induced PD rats. As anticipated, intraperitoneal injection of Rotenone has brought about significant changes in the behavioral parameters as these rats (PD group) exhibited a progressive decrease in motor activity when compared with the control group. A similar observation was done by von Wrangel *et al.* (2015), who has stated that Rotenone injection shows impairment in the rotarod and hanging wire test. These alterations in PD group are due to the degeneration of dopaminergic neurons in the Substantia nigra pars compacta, as observed in the brain neuropathological changes (Alam and Schmidt, 2002). It is also characterized by loss of neuronal cells with decreased intensity of TH staining in the striatal regions. Reduction in dopamine levels in the striatum and oxidative stress causes loss of motor coordination in the Rotenone treated animal (Saravanan *et al.*, 2005). The Rotenone infusion brings about mitochondrial inhibition causing reduced production of ATP, which is necessary for movement and motor co-ordination (Sherer *et al.*, 2003). The effect of galvanic vestibular stimulation

on discrete brain regions of neurotransmitter level, as well as improvement of axial motor function, has been reported earlier. (Samoudi *et al.*, 2012) found that galvanic vestibular stimulation (GVS) improved locomotor activity, measured by performance on a rotarod, and enhanced GABA release in the substantia nigra; however, DA release in the striatum was not significantly affected in Hemi parkinsonism of rats. In this study, dopamine neurotransmitter was reduced after inter peritoneal injection of rotenone and CVS has shown a normalizing effect on dopamine neurotransmitter of the corpus striatum. As a result, it was found to reduce neuronal degeneration. The study also confirms that the vestibular stimulation can trigger the vestibular nucleus in the midbrain and subsequently trigger the motor area of the cerebral cortex, thus bringing change in the neuronal firing via extra pyramidal connections down to the spinal cord. By acting via the vestibulospinal pathway, it is bringing alterations in the motor activity of the animals. However, Rotenone induced behavioral alterations are attenuated on intervention with Caloric vestibular stimulations. These improvements in behavioral changes by CVS are attributed to the prevention of degeneration in striatal neurons, thus restoring the normal dopamine levels in the pars compacta of the substantia nigra.

CONCLUSION

This study confirms that the application of warm water caloric vestibular stimulation enhances dopamine level and motor activity in rotenone-induced PD rats when assessed using Actophotometer. Hence, in future translational research among human subjects can be recommended using caloric vestibular stimulation for enhancement of dopamine and motor activity in Parkinson's disease.

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Conflict of Interest

None.

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