



## Effect of caloric vestibular stimulation in a paraquat-induced parkinsonian mouse model

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### ABSTRACT

The present study was taken up to observe the effect of caloric vestibular stimulation on brain neurochemicals in a paraquat-induced Parkinsonian mouse model. 24 healthy, adult male Swiss albino mice with body weight ranging between 25 - 40g were used in the study. The middle ear cavity of the mice was irrigated with hot (40°C) water. 0.5 ml of water was taken in 5 ml syringe with the needle removed. The ear was irrigated with water drop by drop, using the syringe. Gently the earlobe of mice was shaken. The procedure was continued with the other ear. Acetylcholine, dopamine, serotonin and GABA were estimated. The results of the present study show a positive impact of vestibular stimulation in the management of the Parkinson's disease. Further detailed research is required to understand the mechanism of action behind the performance of vestibular stimulation and could be adopted in the management of Parkinson's disease.



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### INTRODUCTION

The disease-related to the nervous system caused due to the damage of dopaminergic neuron located in the brain is Parkinson's disease (PD) (Sailesh and Archana, 2019). It is a slowly progressive neurodegenerative disease. The aetiology and the mechanism behind the disease are not unveiled yet. But, it is assumed that various environmental related or genetic related factors are the major

contributing factors for PD. Release of reactive oxygen species (ROS) due to increase in oxidative stress leads to a reduction in the production of anti-oxidations further causing mitochondrial damage, and the dysfunction of mitochondria has a link with PD (Jimenez-Jimenez *et al.*, 2014). (Shibeshi *et al.*, 2006), in his study stated that there would be an increase in the formation of Lewy's bodies, his causes the damage to nerve fibers (Shibeshi *et al.*, 2006). In 1000 individuals about 1 to 2 people are suffering from PD. A drug named levodopa is known as a standard traditional drug, accepted and proved to be efficient in treating and managing PD. Unfortunately, this drug too has several side-effects like impairment in cognition and imbalance, leading to poor quality of life of patients with PD (Lee *et al.*, 2015). It is the need of time that there should be some alternative therapy, either drug or technique to treat and manage PD, ensuring minimal to no side effects. Vestibular stimulation is said to be one such tool playing a crucial role in correcting various disorders like obesity, insomnia, anxiety. This system has an association with a brain and mediates the

nervous system related activities, as it consists of tiny receptors in the inner ear involved in the maintenance of equilibrium. At present, the researchers globally are focusing and working in the field of the vestibular system, its functioning and on the areas of its application in correcting the complications arising from mental and physical stress. As research demonstrated that the connection of the vestibular system with the brain affects the whole body of the living being. The studies also proved that stimulation has no detrimental effect and can be applied throughout life. Neurodegenerative disease like PD is also associated with the damage in the connections of interhemisphere as observed in any other neurodegenerative disorder. Vestibular stimulation had proved to improve the interhemisphere connections; this is the positive and significant (Lee *et al.*, 2015). Certain studies had even emphasized that there was enhanced in the motor system functioning by vestibular stimulation when applied in PD patients. Improved performance of rotarod is yet another achievement of vestibular stimulation seen when used in animals the mechanism behind its performance is the release of gamma-aminobutyric acid as a result of vestibular stimulation (Samoudi *et al.*, 2012). PD affects both motor and non-motor systems, justifying the act of motor system defect is subjected to the damage of dopaminergic system and non-motor symptoms due to the effect caused to neurotransmitters, cholinergic neuron damage shows the signs of reduced functions related to cognition (Rizzi and Tan, 2017). On the other hand, the serotonergic system damage or dysfunction in PD leads to weight loss, depression, fatigue, etc., This states the pivot role played by neurotransmitters in PD accounting for the glutaminergic transmission abnormalities too. Researches when tried to study the part of vestibular stimulations and its effects on brain neurotransmitters, had found that on cold water stimulation in rats led to an increased release of acetylcholine from hippocampus and serotonin release was also raised on vestibular stimulations in guinea pigs (Horii *et al.*, 1994). The vestibular system and its effects -altering the levels of neurotransmitters, thus, proves to be beneficial in relieving the negative signs and symptoms in PD patients. In our earlier studies in this area stated very clearly that vestibular stimulations have a positive effect in improving learning, cognition, verbal and spatial memory in animals and human too. The present study was taken up to observe the impact of caloric vestibular stimulation on brain neurochemicals in a paraquat-induced Parkinsonian mouse model.

## MATERIALS AND METHODS

### Animals

24 healthy, adult male Swiss albino mice with body weight ranging between 25 - 40g were used in the study. Mice were housed under standard laboratory conditions with food and water provided *ad libitum*. All studies were conducted in accordance with the Indian National Science Academy guidelines as well as for data management and interpretation, and all efforts were made to minimize the number of animals used and their suffering.

Mice were randomly assigned to four groups.

#### Group 1 (n=6): Control mice

Mice were administered intraperitoneal (i.p.) injections of saline (0.9%) per day.

#### Group 2 (n=6): PD mice

Mice were administered i.p. Injections of paraquat (PQ) (10 mg/kg body wt.) twice weekly for 30 days.

#### Group 3 (n=6): Hot water vestibular stimulation group

Mice were administered i.p. Injections of paraquat (PQ) (10 mg/kg body wt.) (Kasture *et al.*, 2009) twice weekly for 30 days hot water vestibular stimulation on alternate days for 30 days.

#### Group 4 (n=6): Cold water vestibular stimulation group

Mice were administered i.p. Injections of paraquat (PQ) (10 mg/kg body wt.) (Kasture *et al.*, 2009) twice weekly for 30 days cold water vestibular stimulation on alternate days for 30 days.

### Caloric vestibular stimulation

The middle ear cavity of the mice was irrigated with hot (40°C) Water. 0.5 ml of water was taken in 5 ml syringe with the needle removed. The ear was irrigated with water drop by drop, using the syringe. Gently the earlobe of mice was shaken. The procedure was continued with the other ear (Horowitz *et al.*, 2004).

### Outcome measures

#### Estimation of brain neurotransmitters

After 30 days of the experimental period, the animals were fasted overnight and sacrificed by cervical decapitation. The brains were excised immediately, and the brain tissue was homogenized in ice-cold n-butanol solution and used for estimation of biochemical parameters. All the test protocols were carried out between 10:00 am to 12:00 pm. Acetylcholine, dopamine, serotonin and GABA were estimated by using kits and purchased from Sigma Chemical Co (Moron *et al.*, 1979).

### Study setting

The present study was conducted at Little Flower Medical Research Centre, Angamaly, Kerala, India.

### Ethical consideration

The institutional animal ethical committee approved the study of Little Flower Hospital and Research Centre, Angamaly, Kerala, India.

### Data analysis

Data was analyzed by using SPSS 20.0 version. One way ANOVA followed by Tukey HSD post hoc test was applied to observe the significance of the difference between the groups. A probability value of less than 0.05 was considered significant.

## RESULTS AND DISCUSSION

Acetylcholine levels were significantly decreased in group 2 ( $P < 0.001$ ), group 3 ( $P < 0.0006$ ) and group 4 ( $P < 0.001$ ) when compared with group 1. There was significantly higher acetylcholine in group 3 ( $P < 0.0001$ ) and group 4 ( $P < 0.001$ ) when compared with group 2. Acetylcholine levels are not significantly different between group 3 and group 4. Dopamine levels were significantly lower in group 2, group 3 and group 4 ( $P < 0.001$ ) when compared with group 1. Dopamine levels are significantly higher in group 3, group 4 ( $P < 0.001$ ) when compared to group 2. Dopamine levels are significantly higher in group 4 ( $P < 0.001$ ) when compared with group 3. Serotonin levels are significantly higher in group 2 ( $P < 0.001$ ) when compared to group 1. Serotonin levels are significantly lower in group 3 and group 4 ( $P < 0.001$ ) when compared to group 1. Serotonin levels are significantly lower in group 3 and group 4 when compared to group 2 ( $P < 0.001$ ). Serotonin levels are significantly higher in group 4 when compared with group 3 ( $P < 0.001$ ). Glutamate levels are significantly lower in group 2 ( $P < 0.001$ ), group 3 ( $P = 0.0052$ ), group 4 ( $P = 0.0085$ ) when compared with group 1. Glutamate levels were significantly higher in group 3 and group 4 ( $P < 0.001$ ) when compared with group 2. Glutamate levels are not significant between group 3 and group 4. GABA levels are significantly higher ( $P < 0.001$ ) in group 2 when compared with group 1. GABA levels are significantly lower ( $P < 0.001$ ) in group 3 and group 4 when compared with group 1. GABA levels are significantly lower ( $P < 0.001$ ) in group 3 and group 4 when compared with group 2. GABA levels are significantly higher ( $P = 0.0102$ ) in group 4 when compared with group 3. Table 1 Shows,

The present study was undertaken to study the effectiveness of caloric vestibular stimulation on brain neurochemicals in the paraquat-induced

parkinsonian mouse model. Changes in the levels of neurotransmitters as a positive effect was observed from the results of the present study. The studies demonstrated the association of risk of falling in PD patients with the dysfunction of the vestibular system. This is justified by explaining the activation of vestibular afferents by galvanic vestibular stimulation (GVS), causes changes in vestibular input possessing a significant influence on the balancing and posture of an individual. The extrapyramidal structures gets activated by GVS further leading to the increase in the axial motor function; thus, improves postural instability (Kataoka *et al.*, 2015). In PD patients, we notice a higher prevalence of tinnitus, tremor, gait unbalance, risk of falling and dizziness. In this context, the peripheral vestibular system and also the caloric test showed alterations (Bassetto *et al.*, 2008). Pedunclopontine nucleus- thalamic controls the postural sensory integration in PD patients. So, in PD patients, when there is impairment in this section causes impairment in thalamic efferents leading to postural instability (Müller *et al.*, 2013). On the other hand, the anterior bending angle in PD patients is improved by binaural galvanic vestibular stimulation (BVS) (Okada *et al.*, 2015). In Parkinsonian patients, there is a reduction in the excitability of vestibular nuclei and DOPA modulates this act (Pötter-Nerger *et al.*, 2012). The vestibular information processing is impaired in Parkinsonian patients accounting the effect caused by lateral trunk flexion. Partially the impairment is responsible for the postural abnormality of patients (Vitale *et al.*, 2011). The spatial and visual abilities in Parkinson's disease are observed to be affected by dysfunction effects of frontal-parietal systems and frontal-basal ganglionic (Crucian *et al.*, 2000). Vestibular stimulation is a natural treatment for PD, suggested by previous studies (Sailesh *et al.*, 2013). Despite of the fact, that India has a very low prevalence of PD, but its research achievements in the mentioned area are appreciable, and globally it holds the 16<sup>th</sup> rank (2002-2011) (Bala and Gupta, 2013).

The common signs and symptoms observed in PD patients are cognitive disorders, depression, anxiety, apathy, psychosis, suicidal behaviour, anhedonia etc., (Grover and Malhotra, 2015). The performance-based tasks like attention, executive functions and memory is noticed to be worst and poor in the patients with early PD (Adwani *et al.*, 2016), explained by delayed saccade switching while changing the targets one to other. The delay is significantly low in PD patients when compared with normal individuals. This is proof to

**Table 1: Brain neurotransmitter levels in control and intervention groups**

Groups	Group 1	Group 2	Group 3	Group 4	F value
ACH (mole/minute/mg tissue)	0.298667 ± 0.00446219	0.177667 ± 0.00383551	0.244333 ± 0.0263624	0.246333 ± 0.00594792	77.1146
Dopamine (ng/g tissue)	0.2084 ± 0.00587889	0.1191 ± 0.00215438	0.14185 ± 0.00136931	0.1533 ± 0.0020083	765.1223
Serotonin (ng/g tissue)	0.13455 ± 0.000383407	0.14195 ± 0.000967643	0.112 ± 0.000803326	0.13115 ± 0.00122325	1212.5167
Glutamate (ng/g tissue)	0.628667 ± 0.139156	0.333 ± 0.0143852	0.425667 ± 0.0123953	0.441 ± 0.0208135	18.2887
GABA (ng/g tissue)	0.15695 ± 0.000712038	0.1803 ± 0.00186226	0.13925 ± 0.000602494	0.14175 ± 0.001844	1102.8217

(Data was expressed as mean and SD)

explain that not just basal ganglia are involved in the somato-motor loop, but it also requires oculomotor loop related to frontal sub cortical circuit (Joti *et al.*, 2007). PD patients do complain dysfunction of the cardiac autonomic system as they reported reduced blood pressure and the reduced response of heart rate on autonomic stimulation (Varghese *et al.*, 2015). Many Ayurvedic therapies like massages using medicinal oils, natural gait correcting exercises, etc; are in practice to treat and manage the PD disease (Kishore *et al.*, 2010).

Vestibular System plays an important role in regular life, as it contributes to a varied range of functions say from reflexes to the highest level of consciousness and perception. The newest popular therapy is the application vestibular stimulation for developmentally delayed children. The extensive preliminary studies demonstrated that the application of controlled vestibular stimulation through swing serves as an intervention for learning disability and also learnt that it would relieve stress, promotes sleep further improves immunity, relieves cancer pain, and also to treat the endocrine disorders (Varghese *et al.*, 2015). The study conducted in *Wistar albino* rats by application of cold water vestibular stimulation had shown the suppression of the stress-induced changes observed under immunological parameters (Purushothaman *et al.*, 2015). Wherein both cold and hot water vestibular stimulations are proven to be beneficial in maintaining the lipid profile as studied in *Wistar albino* rats (Sadanandan *et al.*, 2015). Hot water caloric vestibular stimulation noted the improvement of cognition in *Wistar albino* rats when induced with scopolamine in the condition of partial amnesia (Gopinath *et al.*, 2015).

## CONCLUSION

The results of the present study show a positive impact of vestibular stimulation in the management of the Parkinson's disease. Further detailed research is required to understand the mechanism of action behind the performance of vestibular stimulation and could be adopted in the management of Parkinson's disease.

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