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Oxidative stress and antioxidant status in rotenone induced rat models of Parkinson's disease

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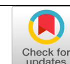
Abstract



Parkinson disease (PD) is involved in the damage of neurons of the brain that secretes dopamine. Understanding the mechanisms involved in Parkinson's disease helps to develop effective management methods. Hence, the present work aimed to unravel the variations, if any, in the parameters of oxidative stress and antioxidant markers between oral and ip rotenone-induced rat model of Parkinson's disease. Male Wistar rats weighing between 250-300g were housed in solid bottomed polypropylene cages under strict veterinary supervision and maintained in rooms with 12hrs light / dark cycle. The randomly selected male Wistar rats were equally divided into four groups, with six rats in each group. The study results provide scientific evidence for marked changes in both the oxidative and anti-oxidant parameters, followed by the ip or po rotenone administration. It is the need of the time to consider the neuroprotective and antioxidant properties while developing the newer treatment modalities for the management of PD.

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INTRODUCTION

Parkinson disease (PD) is involved in the damage of neurons of the brain that secretes dopamine (Sailesh and Archana, 2019). The detailed molecular-level mechanisms of development of PD are still not clear. Oxidative stress was reported to cause damage of the dopaminergic neurons. Complex-I deficiencies in the respiration were reported to involve in the degeneration of the neurons in PD. Further, other factors like insecticides, neurotoxins, pesticides, dopamine (DA) leads to the damage of mitochondria. Oxidative stress has been considerably reported in the development and progress of Parkinson's disease (Hassanzadeh and Rahimmi, 2019; Anderson and Maes, 2014; Caviness *et al.*, 2011; Jimenez-Jimenez *et al.*, 2014; Henze *et al.*, 2005; Dalfó *et al.*, 2005). Though rotenone is the most commonly used model in the development of PD, there are different studies where different routes of administration were used. There were significant differences in histology between the oral and ip administration of rotenone in animal models. However, in our studies, there was no significant difference On selected parameters following different routes of PD induction. Understanding the mechanisms involved in Parkinson's disease helps to develop effective management methods. Hence, the present work aimed to explore any alterations in the levels of oxidative stress and antioxidant markers between oral and ip rotenone-induced rat model of

Parkinson's disease.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250-300g were kept in solid bottomed polypropylene cages under standard conditions of care and maintained in rooms with 12hrs light / dark cycle. The animals were maintained in standard environmental conditions and provided with commercial rat pellet and water *ad libitum*. The randomly selected male Wistar rats were equally divided into four groups, with six rats in each group.

Group I (n = 6): Rats received olive oil (2.0mL/Kg b.w) as vehicle i.p for 21 days.

Group II (n = 6): Rats received rotenone (3 mg/2.0mL/kg b.w) once daily i.p for 21 days.

Group III (n = 6): Rats received 0.5 % HPC (5mL/Kg b.w) once daily p.o for 28 days.

Group IV (n = 6): Rats received rotenone (50 mg/5.0mL/Kg b.w) once daily p.o for 28 days.

Rotenone Treatment

Rotenone was prepared in 100 % Dimethyl sulphoxide (DMSO), diluted with Olive oil to get a concentration of 3 mg/kg b.w. The solution was made fresh every week and vortexed several times before each intraperitoneal injection to avoid the possibility of settling. The rotenone solution and the vehicle olive oil were administered intra-peritoneally once daily at 2 mL/kg.b.w. to PD and control group rats respectively for 21 days. For oral administration rotenone was prepared in 0.5 % HydroxyProyl Cellulose (HPC) to obtain a concentration of 50 mg/kg.b.w. The solution is made fresh every week and vortexed several times before each oral administration to avoid the possibility of settling. The rotenone solution was administered orally at 5 mL/kg.b.w. And the control animals received 0.5 % HPC only for 28 days. All animals were carefully monitored twice daily for clinical and distress signs throughout the experiments. After completing the treatment protocol, the rats were anaesthetized with isoflurane, and the brain was dissected.

Preparation of tissue homogenate

The isolated brain samples were immediately rinsed in ice-cold saline and weighed. A 10% (w/v) tissue homogenate was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The supernatant fraction for the assay of enzymes was obtained by centrifugation of the homogenate at 12,000 rpm for 60 min at 4ºC. Spectrophotometer (Elico, B-200) was used for subsequent assay.

Estimation of LPO

The lipid peroxidation assay was done by following the procedure of Wills (1966). The results were expressed as nmoles MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric acid chromophore. The kit was purchased from Sigma Aldrich, USA.

Estimation of protein carbonyl content

Protein oxidation was evaluated by measuring protein carbonyl content by the method of Levine *et al.* (1990). The protein carbonyl content was expressed as %. The kit was purchased from Sigma Aldrich, USA.

Estimation of Reduced glutathione

The method described by Moron *et al.* (1979) was employed for GSH levels estimation. GSH was used as a standard to calculate the content of GSH, which is expressed as μmol of GSH/mg of protein. The kit was purchased from Sigma Aldrich, USA.

Estimation of Glutathione peroxidase

Glutathione peroxidase activity was measured as per the method described by Rotruck *et al.* (1973) with some modifications. The kit was purchased from Sigma Aldrich, USA.

Estimation of Catalase

The assay of catalase activity was done by the method of Sinha (1972). The activity of catalase was expressed as μmoles of H_2O_2 decomposed /min/mg protein. The kit was purchased from Sigma Aldrich, USA.

Ethical considerations

The Institutional Animal Ethics Committee f Saveetha Medical College approved this study with the IAEC Approval Number: SU/CLAR/RD/014/2015.

Statistical Analysis

Results are expressed as mean and standard error (SE). Statistical significance of the data was determined by one-way analysis of variance (ANOVA). The Student–Newman–Keuls (SNK) posthoc test was used for multiple comparisons to identify sample means that were significantly different from each other. The analysis was carried out using Sigma Plot 13.0 (Systat Software Inc, USA). A p-value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

There were significant changes in the oxidative and antioxidant parameters after the administration of

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Table 1: The effect of rotenone on serum malondialdehyde (MDA) and protein carbonyl (Pr carb) in Wistar rats.

S. No	Parameter	Groups	Mean + SEM
1.	MDA	Con-OO	3.4+ 0.15
		Rot-3-ip	5.6 + 0.17
		Con-HPC	4.9 + 0.20
		Rot-50-po	6.3 + 0.16
2..	Protein carbonyl %	Con-OO	22.0 + 1.04
		Rot-3-ip	41.0 + 1.66
		Con-HPC	25.3 + 0.79
		Rot-50-po	44.3 + 0.45

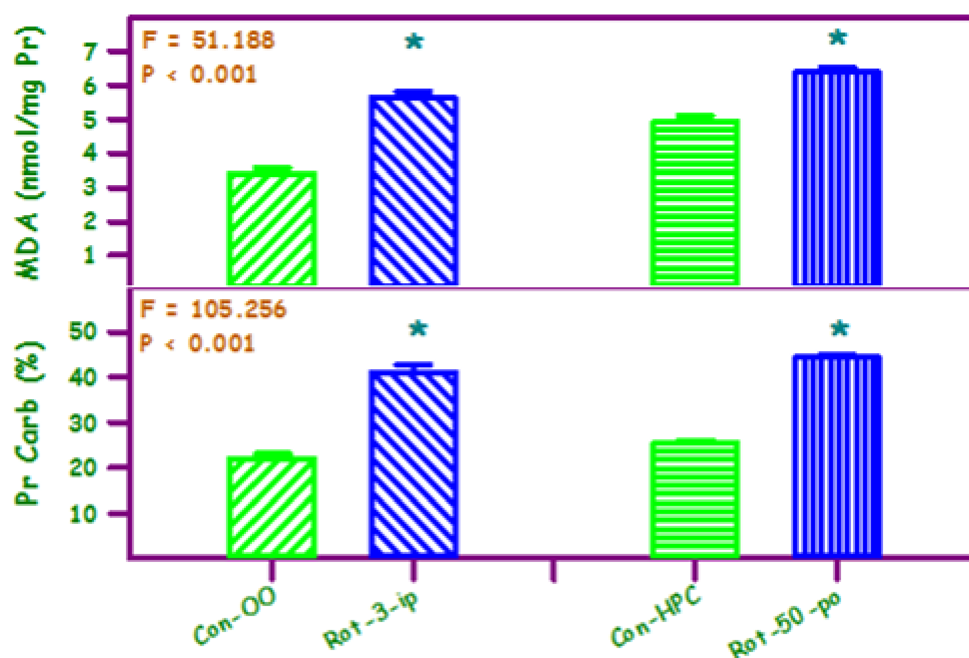

Figure 1: The effect of rotenone on serum malondialdehyde (MDA) and protein carbonyl (Prcarb) in rats. (The values are mean + SE (n = 6 each).
Con = control; OO = olive oil, i.p.; HPC = hydroxypropyl cellulose, p.o.
Rot = rotenone. 3 and 50 are mg/kg dose; Pr = protein.
The 'F' and 'P' values are obtained by one way ANOVA with Student-Newman-Keul's multiple comparison test.
***Significantly different from the respective control groups.)**

rotenone, ip or po. Increased lipid peroxidation (MDA) was observed in the blood of Rot-3-ip

and Rot-50-po compared to the corresponding controls, Con-OO and Con-HPC (Figure 1 and Table 1; $p < 0.001$). Protein carbonyl content was significantly increased in Rot-3-ip and Rot-50-po compared to the corresponding controls, Con-OO and Con-HPC (Figure 1 ; $p < 0.001$). These Alterations could indicate increased oxidative stress caused by rotenone treated rats. The non-enzymatic anti-oxidant parameter Glutathione levels were significantly decreased in Rot-3-ip and Rot-50-po compared to control groups (Figure 2; $p < 0.001$) and the enzymatic anti-oxidant parameters; glutathione peroxidase and catalase levels in the brain striatum were significantly decreased in Rot-3-ip and Rot-50-po compared to control groups (Figure 2 and Table 2; $p < 0.001$). This decrease indicates increased oxidative stress due to rotenone toxicity. Overall, ip and po rotenone administration markedly altered oxidative and anti-oxidant parameters, findings that provide further indications of significant systemic toxicity.

Understanding the underlying mechanism is most crucial in the development of effective therapies. Oxidative stress associated complications are well

Table 2: The effect of rotenone on glutathione peroxidase (GPx), glutathione (GSH) and catalase

S. No	Parameter	Groups	Mean + SEM
1.	GPX	Con-OO	45.2+ 1.0
		Rot-3-ip	21.8 + 1.0
		Con-HPC	43.1 + 0.8
		Rot-50-po	23.9 + 0.8
2.	GSH	Con-OO	1.8+ 0.01
		Rot-3-ip	1.5 + 0.02
		Con-HPC	1.7 + 0.03
		Rot-50-po	1.3 + 0.02
3.	Catalase	Con-OO	11.6 + 0.2
		Rot-3-ip	5.4 + 0.23
		Con-HPC	11.8 + 0.21
		Rot-50-po	5.4+ 0.21

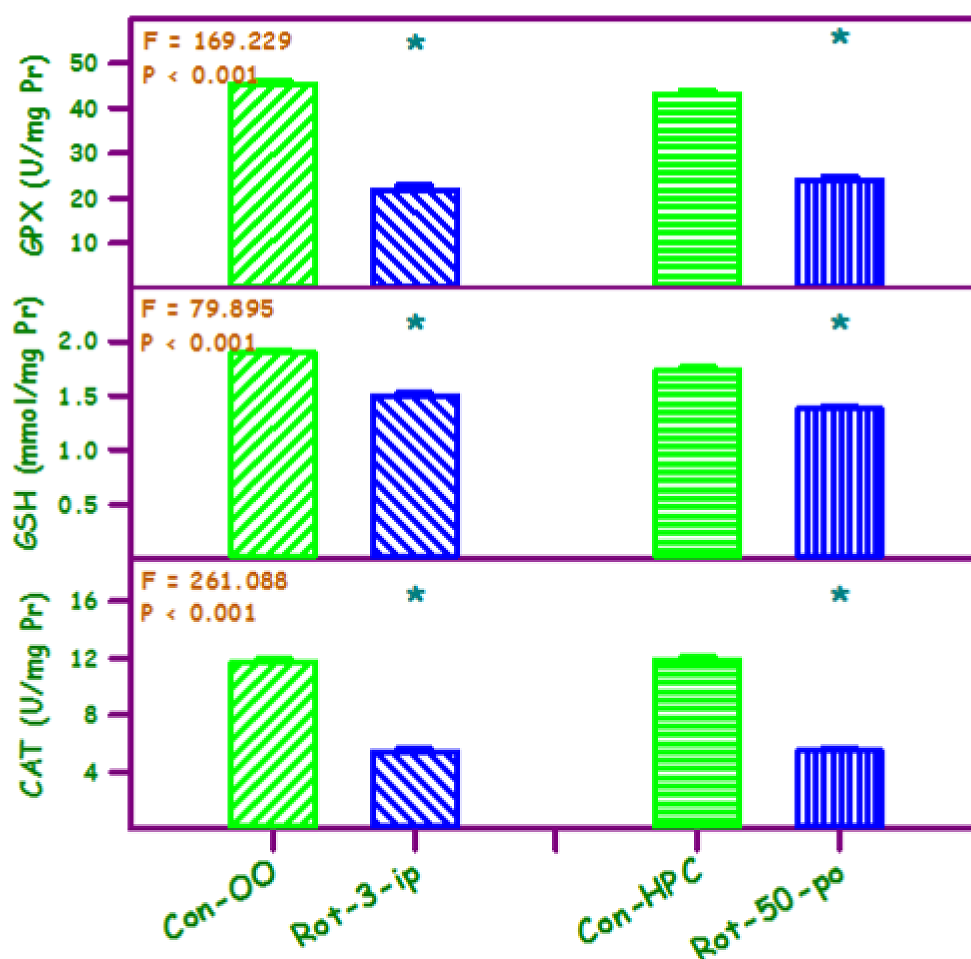

Figure 2: The effect of rotenone on serum glutathione peroxidase (GPx), reduced glutathione(GSH) and catalase (CAT) in rats. (The values are mean + SE (n = 6 each).
Con = control; OO = olive oil, i.p.; HPC =hydroxypropyl cellulose, p.o.
Rot = rotenone. 3 and 50 are mg/kg dose.
The 'F' and 'P' values are obtained by one way ANOVA with Student-Newman-Keul's multiple comparison test.
***Significantly different from the respective control groups)**

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indicated in both animal models as well as in human PD subjects (Sailesh and Archana, 2019).

In recent years, alternative therapies like vestibular stimulation were used in the management of PD. Increased oxidative stress causes excessive release of ROS and causes damage of mitochondria and also causes inflammation of the neurons. These changes together lead to the damage of the neurons secreting dopamine and lead to the PD. The elevated levels of protein carbonyl content and lipid peroxidation products seen in different means of PD induced rats are indicative of severe oxidative stress in PD pathogenesis (Khan *et al.*, 2010; Haleagrahara and Ponnusamy, 2010; Gaballah *et al.*, 2016). Earlier studies reported that there is an increase in the levels of oxidative stress biomarkers in PD (Niedzielska *et al.*, 2016; Johnson *et al.*, 2012; Rajagopalan *et al.*, 2017). The present study aimed to observe whether PD induced with either ip or oral rotenone caused any abrupt changes in the levels of oxidative stress markers such as protein carbonyl, and lipid peroxidation in the serum samples of rats. The results showed a significant elevation of oxidative stress markers (protein carbonyl, lipid peroxidation) and reduced antioxidant status (Catalase, GPx, and reduced glutathione) in both ip and oral rotenone-induced PD rats. Reduced levels of serum catalase and GPx activities could indicate poor antioxidant defense in PD condition (Birben *et al.*, 2012). However, it is noticed that the peripheral alterations leading to oxidative damage were slightly higher in ip than oral rotenone PD, but the difference was statistically significant when compared between the groups. The results of our findings warrant the application of early interventions with drugs that are having higher antioxidant and neuroprotective potentials to ameliorate oxidative stress involved PD complications.

CONCLUSIONS

The study results provide scientific evidence for marked changes in both the oxidative and antioxidant parameters, followed by the ip and po rotenone administration. It is the need of the time to consider the neuroprotective and antioxidant properties while developing the newer treatment modalities for the management of PD.

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