Original Article

Protective Effects Of Resveratrol Against Rotenone-Induced Rat Model Of Parkinson's Disease

Dr. Varadharaju B [1], Dr. Srinivasan Vengadassalapathy [2*], Dr. J. Thirunavukkarasu [3]

[1] Department of Physiology, Saveetha Medical College, Chennai, Tamil Nadu
[2*] Department of Pharmacology, Saveetha Medical College, Chennai, Tamil Nadu
[3] Department of Pharmacology, Saveetha Medical College, Chennai, Tamil Nadu

[*]Corresponding Author
Dr. Srinivasan Vengadassalapathy,
Associate Professor, Department of Pharmacology, Saveetha Medical College, Chennai, Tamil Nadu, India.
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Abstract

This present study compares the effect of resveratrol which is a neuroprotective agent with the ropinirole which is the standard of care in the parkinson's disease.The neuroprotective potential of resveratrol is dependent on its entry and bioavailability in the brain

Materials and Methods: In this study we try to find the combination effect of Resveratrol and Ropinirole together, no study has been done on this. The dose titration study on Resveratrol has not been done too. The rat model of Parkinson's is a reliable model to assess and analyze the drug effects and also the safety profile.

Results and conclusion: This study helps to establish the dose related effect of resveratrol in rotenone induced rat model. Study findings revealed that the medication combination had more neuroprotective effectiveness than the gold standard and individual drug treatments when compared to the rotenone group.

Keywords: Parkinson's Disease, Neuroprotection, Resveratrol, Ropinirole, Rotenone-Induced Rat Model

Introduction

Parkinson's Disease is characterised by motor impairments include resting tremor, stiffness, bradykinesia, and postural abnormalities, according to the Movement Disorder Society (MDS). Additionally, it is linked to pathological signs such the shrinkage of the dopaminergic substantia nigra pars compacta (SNpc) neurons and the development of alpha synuclein in the cytoplasm of the neurons. Additionally, PD is accompanied with neurochemical alterations such as a decline in tyrosine hydroxylase and a fall in striatal dopamine (DA) levels. According to the experimental data, complex I of the mitochondrial electron transport chain (ETC) impairment is a major factor in the aetiology of Parkinson's disease (PD). Ropinirole, the gold standard treatment for Parkinson's disease, and resveratrol, a neuroprotective drug, together have an impact. Some research has been done using in vitro models. However, a rat model is necessary to confirm the improvement post-PD induction in rat models for the neuroprotective mechanism of resveratrol and ropinirole. This research aids in determining Resveratrol's dose-related effects in rotenone-induced rat models. Resveratrol must enter the brain and be bioavailable for it to have any neuroprotective effects.

Experimental animals

The study was approved by Institutional Animal Ethics Committee (Approval number – SU/CLAR/RD/006/2018) and rats were treated in accordance with the guidelines of CPCSEA. The animals were housed in groups of four/cage under appropriate room temperature with water and food given ad libitum.

Experimental design

Male Wistar albino rats (n=56), weighing approximately 200–250 g was used for the study. Total seven groups were assigned with each having 8 animals. After 7 days of acclimation and 15 days was taken for PD to establish fully in the animals. Stock solution of rotenone was prepared using Dimethyl sulfoxide (DMSO) and it was diluted with olive oil to obtain a final concentration of 3 mg/5 ml rotenone. The rotenone solution was made fresh and stored in an amber bottle to avoid light exposure. The solution was vortexed before intraperitoneal injection to eliminate the possibility of settling. 21 days of Drug intervention was done in each of the seven groups. After receiving drug combination therapy for 21 days, the animals were sacrificed. The brains were immediately removed, preserved on ice, and the midbrain and other parts were dissected out using a rat brain atlas. Once it is done, add phosphate buffer solution (4% paraformaldehyde in 0.1M phosphate buffer saline) (PH7.4).

Table 1: Study Design with Treatment groups.

Group details		Drug details	Dose	Total No
I	Control			8
II	Rotenone Parkinson's disease (PD) induced group	Rotenone + DMSO (Dimethyl sulfoxide) through intracranial injection	Rotenone-3 µg dissolved in 2 µL DMSO (Dimethyl sulfoxide)	8
III	Rotenone Induced PD + Resveratrol High dose	Resveratrol daily suspended in water (vehicle), by intragastric gavage.	10 mg/kg Resveratrol suspended in water (vehicle)	8
IV	Rotenone Induced PD + Resveratrol Low dose Group	Resveratrol suspended in water (vehicle), by intragastric gavage.	2.5 mg/kg Resveratrol suspended in water (vehicle)	8
V	Rotenone Induced PD + Ropinirole Group	Ropinirole dissolved in Saline by intragastric gavage.	Ropinirole (Sigma-Aldrich, USA) was dissolved in Saline and at a dose of 1 mg/kg of body weight.	8
VI	Rotenone Induced PD + Resveratrol Low dose Group + Ropinirole	Resveratrol suspended in water (vehicle) + Ropinirole dissolved in Saline. Both by intragastric gavage	2.5 mg/kg Resveratrol suspended in water (vehicle) + Ropinirole dissolved in Saline and at a dose of 1 mg/kg of body weight.	8
VII	Rotenone Induced PD + Resveratrol High dose Group + Ropinirole	Resveratrol suspended in water (vehicle)+ Ropinirole dissolved in Saline. Both by intragastric gavage	10 mg/kg Resveratrol suspended in water (vehicle)+ Ropinirole dissolved in Saline and at a dose of 1 mg/kg of body weight.	8

Histopathological Assessment

Wistar rats had intracardial perfusion with physiological saline as a pre-rinse solution and then fixative solution of 10% neutral-buffered formalin (NBF). The corpus striatum portion of the brain slice was taken out and post-fixed with 10% neutral-buffered formalin (NBF) for around 48 hours. Following fixation, tissues were dehydrated in progressively higher alcohol concentrations, fixed in paraffin wax, and sectioned into 5-7 m thick pieces using

a rotary microtome. Hematoxylin and eosin staining is then applied to the paraffin slices (Bancroft and Gamble 2008). Under the BX51 Olympus multi head microscope, the corpus striatum of brain tissue was investigated for histopathology.

Immunohistochemical Analysis

The sections of corpus striatum (approximately 0.20 mm relative to bregma) was dissected by microtome and were rinsed in PBS and incubated in 1% H2O2 for 30 min to block the endogenous peroxidase activity. After washing in PBS, the sections were incubated in blocking serum (10% normal horse serum and 0.1% Triton X-100 in PBS) for 60 min, followed by incubation in anti-TH mouse monoclonal antibody solution (1:500) for 24 h at room temperature. The sections were then incubated for 1 h in biotinylated anti-mouse IgG secondary antibody (1:300). The sections were subsequently incubated with avidin-biotin peroxidase complex for 1 h at room temperature. The immunoreactivity was visualized by Incubating the sections consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.02% H2O2 in 50- mM Tris buffer (pH, 7.6) for 3 min.

Hematoxylin and Eosin (H&E) Staining

Histopathological images of corpus striatum stained with hematoxylin and eosin (H&E). (A) Vehicle control (B) PD-1 mg/kg rotenone (C) PD-2 mg/kg rotenone

Determination of Neurotransmitter:

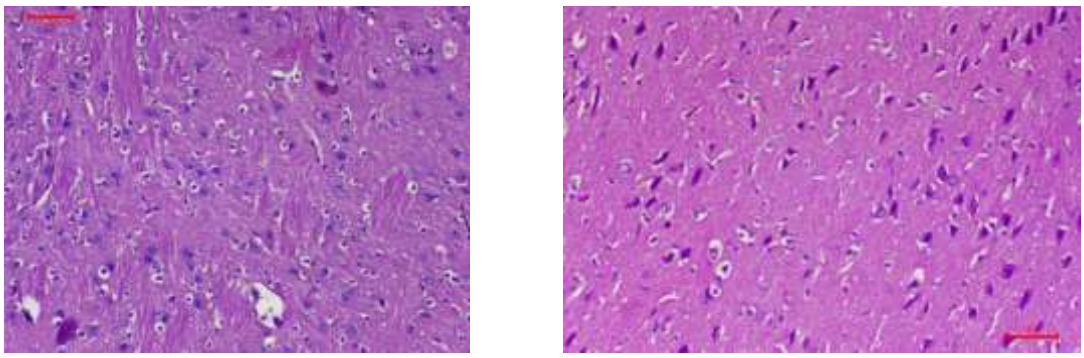
Dopamine in corpus striatum of the rat brain. Dopamine level was estimated by comparing with standard dopamine by measuring the fluorescence intensity using spectroflourimeter.

Results:

Hematoxylin and Eosin (H&E) Staining

Histopathological images of corpus striatum stained with hematoxylin and eosin (H&E). (A) Vehicle control (B)PD-3 mg/kg rotenone (scale bar 20 mm). When compared to gold standard and individual drug combination treatment groups, the resveratrol high dose and the ropinirole drug combination group showed the greatest neuroprotective effect against rotenone toxicity through reversal of pathological alterations and structural repair. The resveratrol high dose and the ropinirole are thus appropriate and effective choices for neuroprotection, according to the findings of histopathology employing H and E staining. (Figure 1)

Figure 1: Hematoxylin and Eosin (H&E) Staining



A B

IL-1 IHC Immunohistochemistry

Substantia nigra: (Fig 1,2,)

- Percentage of cells showing positivity: 70 %
- Average intensity of staining: Strong.
- Pattern of staining: Cytoplasmic and Nuclear.

Striatum: (Fig 3,4)

- Percentage of cells showing positivity: 60 %
- Average intensity of staining: Strong.
- Pattern of staining: Cytoplasmic and Nuclear.

IL-1 IHC Immunohistochemistry

Fig 1 Fig 2

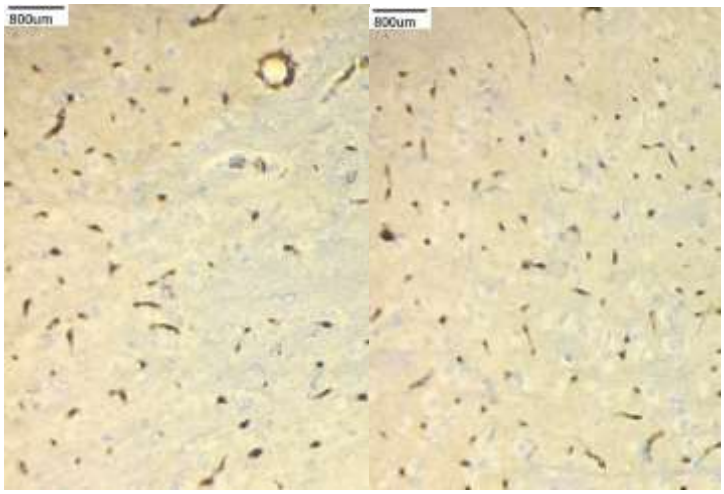
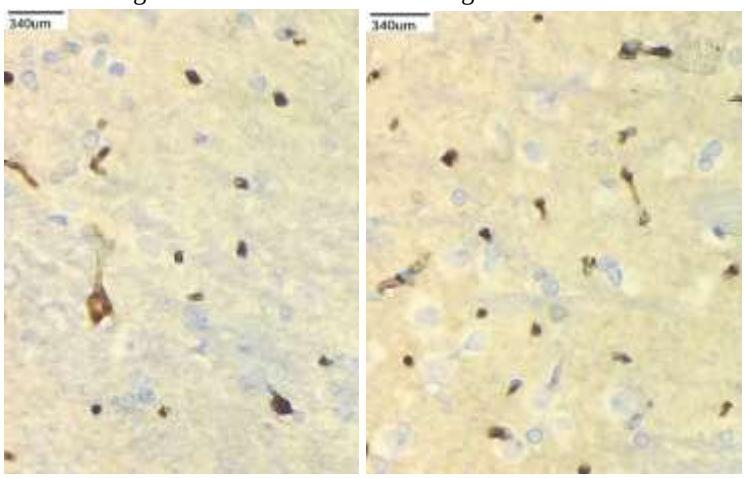


Fig 3 Fig 4



TNF- α IHC Immunohistochemistry

Substantia nigra: (Fig 5,6,7,8)

- Percentage of cells showing positivity: 50 %
- Average intensity of staining: Strong.
- Pattern of staining: Cytoplasmic.

Fig:5 Fig:6

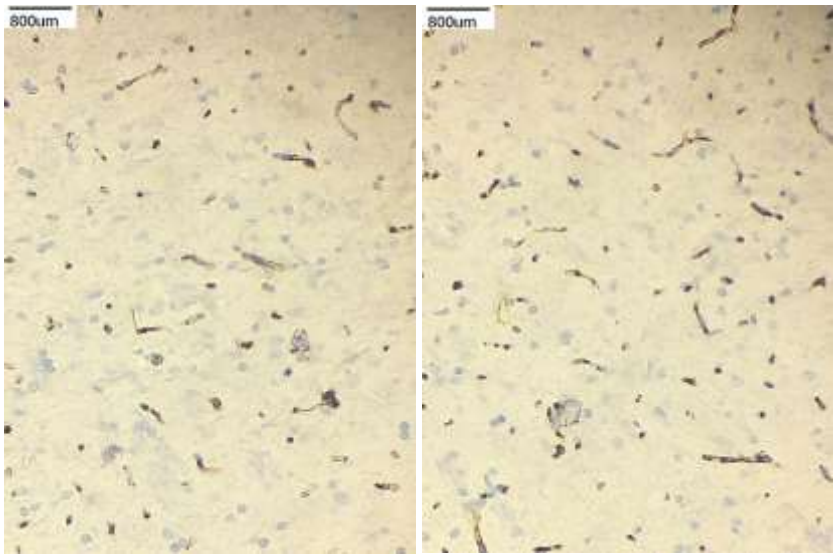
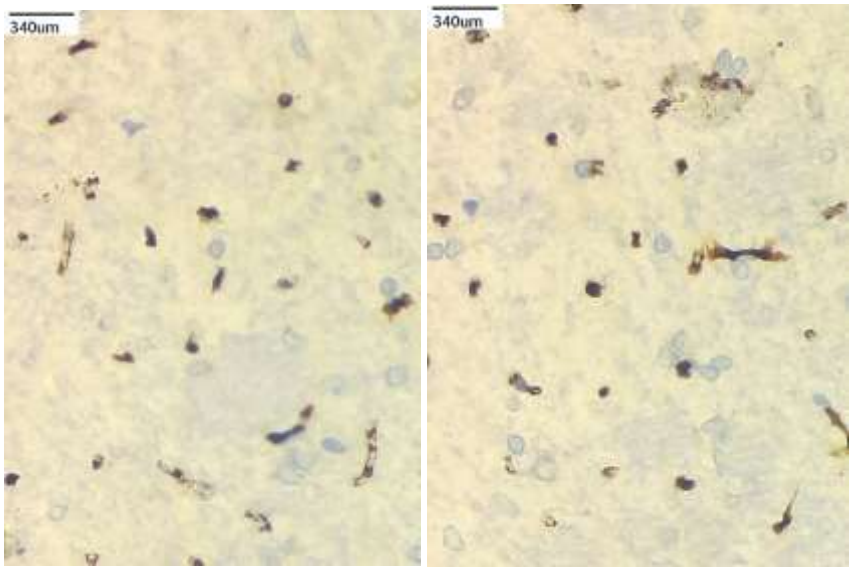


Fig:7 Fig:8



Striatum: (Fig 9,10,11,12)

- Percentage of cells showing positivity: 25 to 30 %
- Average intensity of staining: Strong.
- Pattern of staining: Cytoplasmic.

Fig:9 Fig:10

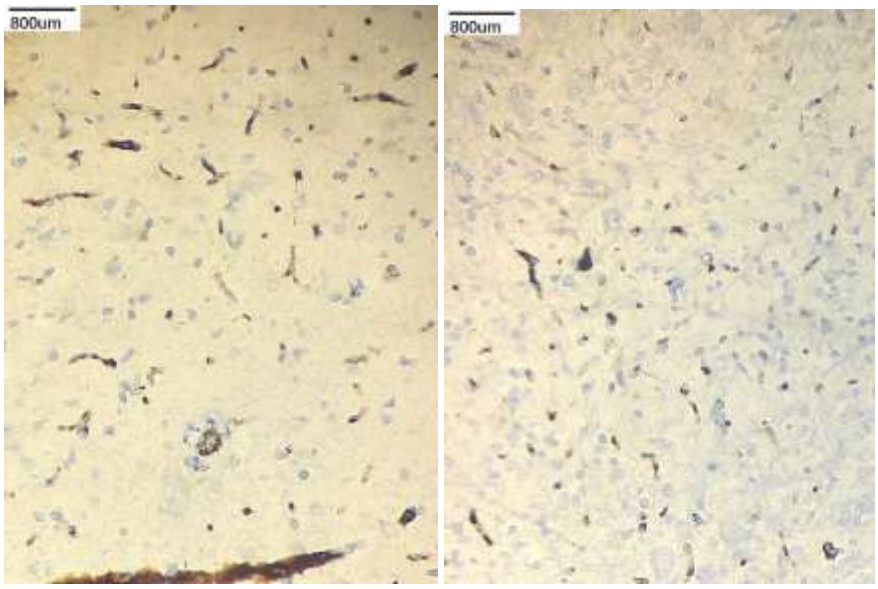
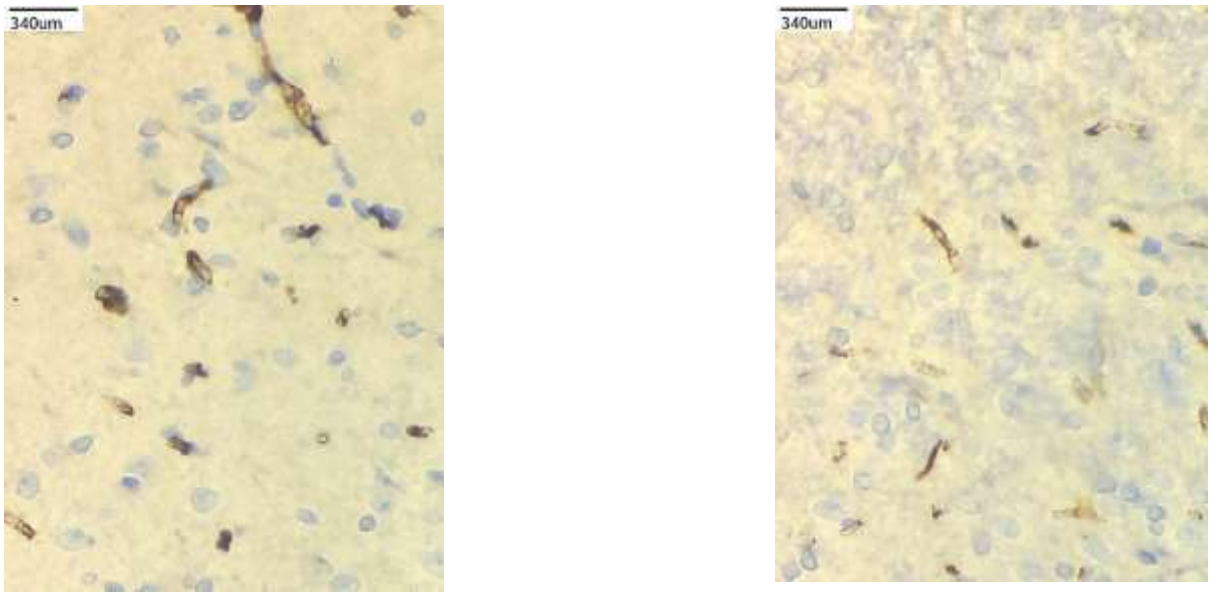


Fig:11 Fig:12

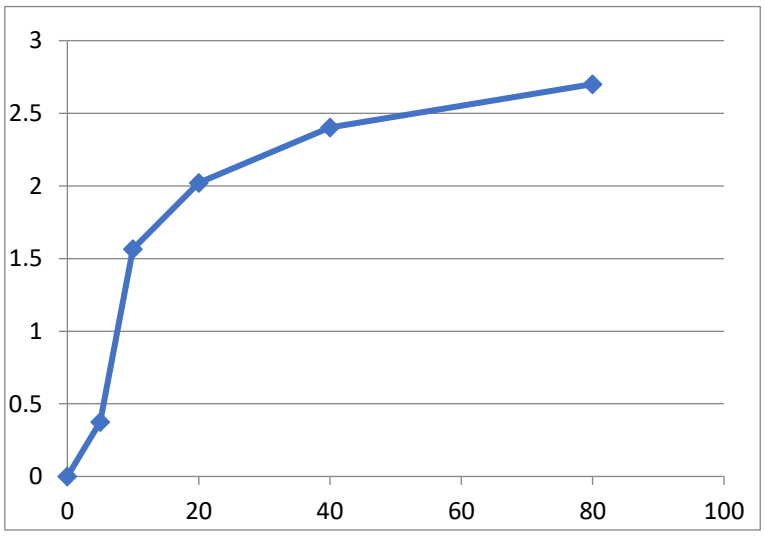


Effect of resveratrol on serum dopamine level

Compared to control, concentration of dopamine in the serum were significantly ($p<0.005$) reduced in Rotenone induced rats. However, treatment with Resveratrol (10mg/kg) increased dopamine near to that of the control group ($p<0.005$) while combination of Resveratrol and Ropinirole treated to rotenone induced rats efficiently increased the levels of dopamine. (Table 2)

Table 2: Effect of resveratrol on serum dopamine level

STD CONC	STD OD	
0	0	
5	0.375	
10	1.566	
20	2.021	
40	2.402	
80	2.701	
S.No	**OD**	**pg/ml**
1	0.6	**5.94**
2	0.815	**8.07**
3	0.767	**7.59**
4	0.774	**7.66**
5	0.591	**5.85**
6	0.557	**5.51**
7	0.696	**6.89**
8	0.673	**6.66**
9	0.717	**7.10**
10	0.625	**6.19**



Discussion:

Autophagy, which helps clear up lewy bodies filled with misfolded -synuclein proteins, has been found to be greatly enhanced by the medication combination. Numerous studies have shown that autophagy is a crucial process for clearing out dysregulated protein aggregates in PD. Thus, autophagy failure is crucial to the aetiology of Parkinson's disease. Additionally, it has been suggested that encouraging autophagy when autophagosome biogenesis is impaired might be a viable treatment approach to treat Parkinson's disease (PD). Our study unequivocally shows that the resveratrol high dose and the ropinirole drug combination is more suitable as a therapy approach for PD's -synuclein proteinopathy. According to several studies, microglia and activated astrocytes release proinflammatory cytokines that lead to neuroinflammation, one of the key features of the pathophysiology of PD. Additionally, neuroinflammation has been thought of as a possible therapeutic target for the treatment of Parkinson's disease. Our study has demonstrated the effectiveness of the resveratrol high dose and the ropinirole drug combination by showing that treatment with the drug combination significantly lowers the levels of IL1, TNF- α IHC Immunohistochemistry staining.

Conclusion:

When compared to the rotenone group, histopathology results suggested that the drug combination has better neuroprotective efficacy than gold standard and individual drug treatments. This is demonstrated by the reversal of pathological changes with little to no lewy bodies, distorted, or degenerated cells. Moreover, a biochemical analysis clearly demonstrated improvements in the amount of autophagy, the level of glutathione, the level of dopamine, and the degree of neuroinflammation. Additionally, the medication combinations' histology data demonstrated reversal of pathological alterations. Estimation of dopamine level in the in vitro rotenone model

revealed that sufficient dopamine levels are maintained by the resveratrol high dose and the ropinirole drug combination group compared to other groups.

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