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Beneficial effect of antidepressants against rotenone induced Parkinsonism like symptoms in rats

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ABSTRACT

Parkinson's disease is a second most common age-related neurodegenerative disorder characterized by the loss of DA neurons of SNpc region of the midbrain. Neurotransmitter dysfunction is involved in the pathogenesis of PD. Antidepressants like venlafaxine and sertraline expected to improve Parkinsonism like symptoms by modulating the levels of various neurotransmitters. The neuroprotective role of antidepressants is well explored in various CNS disorders. Therefore, this study was designed to explore and compare the mechanistic role of different antidepressants (venlafaxine and sertraline) against rotenone induced Parkinsonism like symptoms in rats. Rats were administrated with rotenone (1.5 mg/kg/day; s.c.) daily for a period of 28 days. Venlafaxine (10 and 20 mg/kg; p.o.), sertraline (10 and 20 mg/kg; p.o.) and Levodopa combination with Carbidopa (10 mg/kg; p.o.) were administered daily starting from 7th day one hour prior to rotenone administration. Behavioral parameters (body weight, rotarod, grip strength, narrow beam walk and open field) were assessed on weekly basis. On 28th day, animals were sacrificed and striatum were isolated for biochemical (LPO, GSH and nitrite), neuroinflammatory (TNF-α, IL-1β and IL-6), neurochemical (DA, NE, 5-HT, GABA, Glutamate, DOPAC, HVA and 5-HIAA) and mitochondrial complex-I estimation. Rotenone administration significantly reduced body weight, motor coordination, oxidative defense, increased pro-inflammatory mediators and decreased level of catecholamines. Pretreatment with venlafaxine and sertraline significantly attenuated the alteration in behavioral, oxidative stress, neuroinflammatory, mitochondrial and catecholamines level in striatum. The study provides a hope that these drugs could be used as adjuvant therapy in the management and treatment of PD.

1. Introduction

Parkinson's disease (PD) is second most common age related neurodegenerative disorder affecting approximately 6 million people worldwide. The prevalence of PD increases with age, affecting about 1–2% adults above the age of 60 years and 4% of above the age of 80 years [31,56,51]. PD is hypokinetic, late-onset, progressive neurodegenerative movement disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNPc) and development of fibriller cytoplasmic inclusions containing α-synuclein and ubiquitin [13,57,52]. PD is presented with four primary motor manifestations: tremor at rest, rigidity, bradykinesia (or slowing of movement), and postural abnormalities. Initially, patients show one or two of the classic signs of the PD [59].

Alteration in neurotransmitters homeostasis, mitochondrial dysfunction and oxidative stress are key factor underlying pathogenesis of PD. The neuronal loss seen in SNpc of PD patients is associated with mitochondrial dysfunction and high level of oxidative damage to the macromolecules including DNA, proteins and lipids. Dopaminergic neurones are particularly sensitive to oxidative stress because the metabolism of dopamine generates high levels of reactive oxygen species (ROS). ROS are usually detoxified in a healthy cell; however, an imbalance between ROS production

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; CMC, carboxy-methyl-cellulose; CNS, Central nervous system; Con, control; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IL-1β, Iinterleukin-1β; IL-6, interleukin-6; LPO, lipid peroxidation; MDA, malondialdehyde; NE, nor-epinephrine; NO, nitric oxide; PD, Parkinson's disease; ROS, reactive oxygen species; Rot, rotenone; rpm, rotation per minute; Ser, sertraline; SNPc, substantia nigra pars compacta; SNRI, serotonin-nor epinephrine reuptake inhibitors; SSRI, selective serotonin re-uptake inhibitors; TNF-α, tumor necrosis factor-α.

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and detoxification can quickly lead to cell toxicity [48]. Altered level of neurotransmitters specifically dopamine (DA) in striatum contributes to the behavioral alteration, impaired motor coordination and striatal dysfunctioning [7]. Alteration in DA level is responsible for glutamate mediated excitotoxic cell neuronal death [7]. Overactivation of *N*-methyl-D-aspartate receptors (NMDARs) is responsible for striatal excitotoxicity, specifically through NR2B subunit [34].

Rotenone is a highly lipophilic in nature and specifically inhibits mitochondrial complex I of electron transport chain. The neurodegeneration produced by rotenone is slow and progressive that ensures the use of this pesticide as model to study neuroprotective strategies [50,45]. Rotenone has been used broadly as a classic mitochondrial poison in in-vitro and in-vivo models [39]. According to reports available, rotenone exposure systemically or subcutaneously reproduces the symptoms of PD [50] by selectively degenerates dopaminergic neurons [3].

Sertraline and venlafaxine are well known for their use in the treatment and management of depression and these drugs are expected to improve Parkinsonism like symptoms by increasing the level of these neurotransmitters induced by Rotenone. Studies have evidenced that antidepressant drugs also upregulates the gene expression and activity of antioxidant enzymes [32,18].

There is no available clinical therapy that can limit or halt the neurodegeneration in PD. Current therapy is only palliative, leading to temporarily limited improvement of clinical symptoms and produces side effects [47]. None of the studies investigated the effect of antidepressants against rotenone induced model of PD. Therefore, this study was designed to add new details to the understanding of PD associated neuropsychiatric symptoms and to explore and compared the mechanistic role of different antidepressants (venlafaxine and sertraline) against rotenone induced Parkinsonism like symptoms in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g (4–5 month old), were procured from Central Animal House facility of ISF College of Pharmacy, Moga, Punjab, India were used in the study. Animals were housed in group of three, in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water *ad libitum*. Animals were acclimatized to laboratory conditions before experimentation. The protocol was reviewed and approved by the Institutional Animal Ethics Committee ISFCP/IAEC/CPCSEA/P250 and the animal experiments were carried out in accordance with the Indian National Science Academy Guidelines for use and care of animals.

2.2. Experimental design (Fig. 1)

2.2.1. Drugs and treatment schedule

The following drugs were used in this study. Rotenone (Sigma Chemicals, St Louis, Missouri, USA) (1.5 mg/kg, s.c.) dose dissolved in sunflower oil [3,49] daily for a period of 4 weeks (28 days) . Venlafaxine (10 and 20 mg/kg; p.o.), sertraline (10 and 20 mg/kg; p.o.) (Albro Pharmaceutical Pvt. Ltd. Muktser, Punjab, India) were suspended in 0.5% (v/w) sodium carboxy-methyl-cellulose solution and levodopa in combination with carbidopa (10 mg/kg) administered by the oral route for 21 days starting from 7th day one hour prior to rotenone administration. Doses of venlafaxine and sertraline were selected on the basis of earlier studies [24,26,25]. The study was conducted in several phases, involving the following treatment groups (Total number of animals = 56 and n = 8) (Table 1).

Table 1
Treatment group.

Group1	Control (Sunflower oil + CMC)
Group 2	Rotenone (1.5 mg/kg, s.c.) + CMC for 28 days
Group 3	Rotenone (1.5 mg/kg, s.c.) + Sertraline (10 mg/kg, p.o.)
Group 4	Rotenone (1.5 mg/kg, s.c.) + Sertraline (20 mg/kg, p.o.)
Group 5	Rotenone (1.5 mg/kg, s.c.) + Venlafaxine (10 mg/kg, p.o.)
Group 6	Rotenone (1.5 mg/kg, s.c.) + Venlafaxine (20 mg/kg, p.o.)
Group 7	Rotenone (1.5 mg/kg, s.c.) + Levodopa + Carbidopa (10 mg/kg, p.o.)

2.2.2. Induction of Parkinson's disease

PD was induced by the chronic administration of rotenone (1.5 mg/kg; s.c.) dose dissolved in sunflower oil to rats for a period of 28 days. All the behavioral parameters were assessed every week i.e. on day 14, 21, 28).

2.2.3. Measurement of body weight

The body weight of animals was recorded on the first and last day of experiment. Percentage change in body weight was calculated using formula-

$$\frac{\text{Bodyweight(1st day)} - \text{Bodyweight(28th day)}}{\text{Bodyweight(1st day)} \times 100}$$

2.3. Behavioral assessments

2.3.1. Open field test

Open field test is used to monitor spontaneous locomotor activity using wooden, rectangular, light brown colored open field apparatus measuring 100 × 100 × 40 cm. The floor of the apparatus was divided into 25 rectangular squares by pencil lines. The experimental room was illuminated by 40 W white bulb located 150 cm above the test apparatus. Animal was placed in the centre of apparatus 2 h later, after giving a single exposure to apparatus and number of squares cross/10 min number of grooming/10 min and number of rearing/10 min by animal were recorded. Each crossing was considered if the animal puts all the four paws in another square. After each trial, apparatus was cleaned properly. Total locomotor activity/10 min was calculated by the addition of number of squares crossed, number of grooming and number of rearing [53].

2.3.2. Rotarod activity

The motor coordination and grip performance of the animals were evaluated using the rotarod apparatus. The rats were exposed to a prior training session to habituate them to rotarod performance. Rats were placed on a rotating rod having a diameter of 7 cm (speed 25 rpm). The cut off time was 180 s and the average time of the fall was recorded [25]. It was commercially available from Medicraft INCO, Ambala, Haryana, India, consisting of two or three or four compartments of 75 mm width each with a rotating rod of 25 mm diameter with speeds of 5, 10, 15, 20 and 25 rpm and time interval counter in each compartment. Rats were placed individually on a rotating rod with a diameter of 7 cm (speed 25 rpm). The cut off time was 180 s [23].

2.3.3. Grip strength measurement

Grip strength of the fore limbs was measured using digital grip force meter (DFIS series, Chatillon, Greensboro, NC, USA). The rat was positioned to grab the grid with the fore limbs and was gently pulled to record the grip strength [1]. The grip strength was recorded in Kgf.

2.3.4. Beam-crossing task

This task requires an animal to walk on across a narrow wooden beam, measuring its motor coordination ability. The beam consisted of two platforms (8 cm in diameter) connected by a wooden