

Short communication

**THE NEUROPROTECTIVE EFFECT OF *Withania somnifera* ROOT EXTRACT IN MPTP-INTOXICATED MICE: AN ANALYSIS OF BEHAVIORAL AND BIOCHEMICAL VARIABLES**

SRINIVASAGAM RAJA SANKAR<sup>1\*</sup>, THAMILARASAN MANIVASAGAM<sup>2</sup>, ARUMUGAM KRISHNAMURTI<sup>1</sup> and MANICKAM RAMANATHAN<sup>3</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine, Annamalai University, Annamalainagar, 608 002, India, <sup>2</sup>Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, 608 002, India, <sup>3</sup>Department of Surgery, Faculty of Medicine, Annamalai University, Annamalainagar, 608 002, India

**Abstract:** We studied the influence of *Withania somnifera* (Ws) root extract (100 mg/kg body weight) on parkinsonism induced by 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP; i.p, 20 mg/kg body weight for 4 days), via the analysis of behavioral features and the oxidant-antioxidant imbalance in the midbrain of mice. A significant alteration in behavior, increased levels of thiobarbituric acid reactive substance (TBARS), and increased activities of superoxide dismutase (SOD) and catalase (CAT) were noticed in this region of brain in MPTP-treated mice. Oral treatment with the root extract resulted in a significant improvement in the mice's behaviour and antioxidant status, along with a significant reduction in the level of lipid peroxidation. The results indicated that at least part of the chronic stress-induced pathology may be due to oxidative stress, which is mitigated by Ws. Further studies are needed to assess the precise mechanism to support the clinical use of the plant as an anti-parkinsonic drug.

**Key words:** MPTP, *Withania somnifera*, TBARS, Antioxidants, Behaviour, Midbrain

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\* Author for correspondence; e-mail: [anatomysrs@yahoo.com](mailto:anatomysrs@yahoo.com), tel: + 91-4144-239645, fax : + 91-4144-238145

Abbreviations used: CAT – catalase; MPTP – 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; PD – Parkinson's disease; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances; Ws – *Withania somnifera* root extract

## INTRODUCTION

Parkinson's disease (PD) is an age-related progressive neurodegenerative disease, which is characterized by resting tremors, rigidity, postural abnormalities, particularly stooped posture, and difficulty or failure to execute willed movements, i.e. bradykinesia, akinesia and festinating gait [1-3]. Parkinson's disease occurs due to destruction of nigral dopaminergic neurons resulting in dopamine depletion in the striatum, mainly mediated by oxidative stress and mitochondrial dysfunction [3]. Agents with the ability to interfere with the synthesis, release, uptake, metabolism or drug receptor interaction of dopamine have been shown to alter the course of experimental parkinsonism [4]. Many of the neurotoxic agents that have been used to induce PD in animal models are mitochondrial electron transport chain inhibitors: 6-hydroxy dopamine, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), rotenone and paraquat (pyridine-containing herbicides) [5]. MPTP, a potent neurotoxin, induces Parkinson's disease in various experimental animals including monkeys, mice, cats, dogs, rats and goldfish [6]. The induced neurochemical, behavioural and histopathological alterations very closely replicate the clinical symptoms of PD patients [7].

Various behavioral tests are used as indices to measure the movement impairments in MPTP-induced animal models [8]. They closely mimic PD patient clinical symptoms, such as akinesia, rigidity and gait disturbance. MPTP is known to induce oxidative stress and mitochondrial dysfunction, leading to dopaminergic depletion in the striatum [9].

Drugs with antioxidant properties have been evaluated for their neuroprotective effects against parkinsonian or dopaminergic neurotoxins [9]. *Withania somnifera* (Dunal), popularly known as Indian Ginseng, belongs to the family Solanaceae, commonly known as Ashwagandha, a subtropical undershrub. Bhattacharya *et al.* [10] reported that glycowithanolides (the active principles of Ws) alter the cortical and striatal antioxidant enzyme activities (SOD, CAT and Glutathione peroxidase) in rats. A 30-day treatment with *Withania somnifera* root produced a significant decrease in LPO, and an increase in both SOD and CAT, indicating that Ashwagandha root powder possesses free radical scavenging activity [11]. This experiment is an attempt to study the curative effect of Ws root extract on MPTP-induced parkinsonism by evaluating behavioral differences and the oxidant-antioxidant imbalance.

## MATERIALS AND METHODS

### Animals

Inbred adult male Albino mice (30-35 g) from the Institute colony were used in this study. The animals were kept in a 12-h light/dark cycle, at 22°C and 60% humidity, with food and water *ad libitum*. The experimental protocols met with the National Guidelines on the *Proper Care and Use of Animals in Laboratory Research* (Indian National Science Academy, New Delhi, 2000) and were

approved by the Animal Ethics Committee of the Institute (Approval No. 164/08-2002).

### Chemicals

MPTP, thiobarbituric acid (TBA), reduced glutathione and 3,5-dithio-bis-nitrobenzoic acid (DTNB) were obtained from Sigma chemical Co. (St. Louis, MO. USA.). All the other reagents were of analytical grade and were obtained locally. The commercially available powdered root of Ws was obtained from the Indian Medical Practitioners Cooperative Society, Adyar, Madras, India. Its aqueous suspension was used in this study.

### Experimental design

The mice were divided into four groups of six animals each. The first group of mice was kept as a control. The mice of the second group received injections of MPTP (i.p., 20 mg/kg body weight), one each on 4 consecutive days. The third group received MPTP (i.p., 20 mg/kg body weight) [12], one each on 4 consecutive days, followed by oral administration of Ws [12] for 28 days. The fourth group was treated with Ws orally for 28 days. At the end of the experiment (28 days after the first MPTP injection), the animals were analysed in behavioral studies such as the Rotarod test [8], Hang test [13] and stride length measurement [14]. The mice were killed by cervical dislocation on the following day, and their brains were dissected via the method described by Glowinski and Iversen [15] to procure the midbrains for the analyses of TBARS [16], SOD [17], CAT [18] and protein [19].

### Statistical analysis

All the data was expressed as the means  $\pm$  SD of a number of experiments ( $n = 6$ ). The statistical significance was evaluated by the one-way analysis of variance (ANOVA) using SPSS version 10.0 software, and individual comparisons were obtained using Duncan's Multiple Range Test (DMRT). Values were considered statistically significant if not sharing a common superscript letter or symbol. All the results proved to be statistically significant at  $p < 0.05$ .

## RESULTS

Fig. 1 shows that the MPTP injection significantly ( $p < 0.05$ ) reduced the retention time on the rod (group II) relative to the controls. The administration of Ws (group III) to the lesioned animals significantly increased the retention time ( $p < 0.05$ ). There was no change seen for the animals treated with Ws alone relative to the controls.

Fig. 2 elucidates the significant reduction ( $p < 0.05$ ) in the hang time of the MPTP-injected animals (group II) relative to the controls. The administration of Ws (group III) to the lesioned animals significantly increased the retention time ( $p < 0.05$ ). The group treated with Ws alone did not show any difference from the control group.

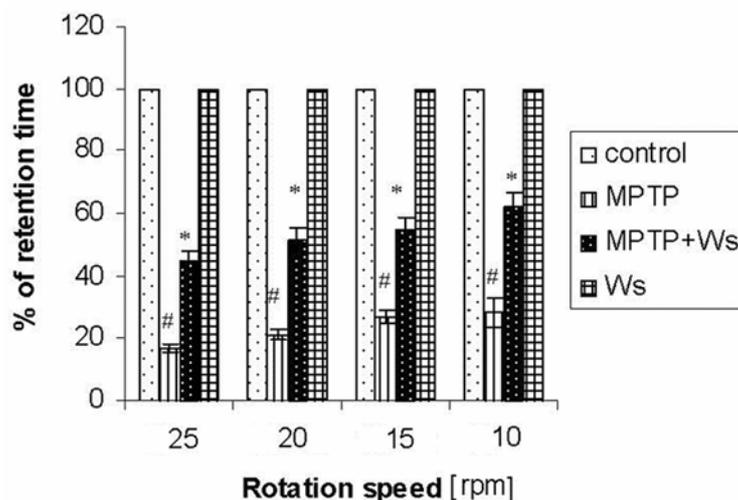


Fig. 1. Variations in rotarod performance at various speeds (25 rpm, 20 rpm, 15 rpm, 10 rpm) measured as retention time (sec) in the control and experimental mice. Percentage values are expressed as means  $\pm$  SD. n = 6, #p < 0.05 vs. control, \*p < 0.05 vs. MPTP.

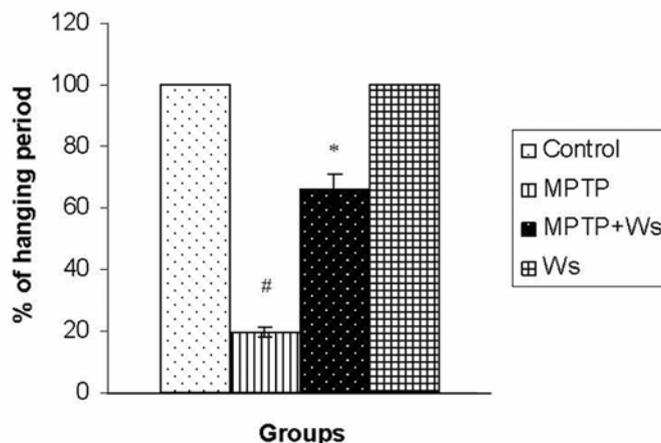


Fig. 2. Variations in the neuromuscular strength measured in terms of relative percentage hang time in the control and experimental mice. Percentage values are expressed as means  $\pm$  SD. n = 6, #p < 0.05 vs. control, \*p < 0.05 vs. MPTP.

Tab. 1 shows the mean stride length differences (for both the fore and hind limb) in the control and MPTP- and Ws-treated groups. The step difference for both significantly ( $p < 0.05$ ) decreased in the MPTP-treated group relative to the controls. Treatment with Ws (group III) significantly ( $p < 0.05$ ) improved this factor relative to the MPTP-intoxicated mice.

Tab. 1. Mean forelimb and hindlimb stride lengths.

Groups	Forelimb paw stride length [cm]	Hindlimb paw stride length [cm]
Control	7.14 ± 0.5 <sup>a</sup>	6.63 ± 0.47 <sup>a</sup>
MPTP	5.43 ± 0.41 <sup>b</sup>	4.83 ± 0.36 <sup>b</sup>
MPTP+ Ws	6.4 ± 0.49 <sup>c</sup>	6.01 ± 0.43 <sup>c</sup>
Ws	7.6 ± 0.6 <sup>a</sup>	6.8 ± 0.54 <sup>a</sup>

Values are expressed as means ± SD (n = 6). Values not sharing a common superscript letter differ significantly at  $p < 0.05$  (DMRT).

Tab. 2 depicts the levels of TBARS and activities of SOD and CAT in the midbrain of mice from the control and experimental groups. The lipid peroxidation levels and activities of SOD and CAT in the midbrain were significantly elevated in MPTP-treated animals (group II) relative to the controls. The administration of Ws to MPTP-treated mice (group III) tends to bring the TBARS, SOD and CAT values to near-normal levels. No significant changes were found in mice treated with Ws alone (group IV).

Tab. 2. Changes in the levels of TBARS and the activities of SOD and CAT in the control and experimental mice.

Groups	TBARS [nmoles/g]	SOD [U <sup>A</sup> /mg protein]	CAT [U <sup>B</sup> /mg protein]
Control	1.51 ± 0.11	0.75 ± 0.06 <sup>a</sup>	0.56 ± 0.04 <sup>a</sup>
MPTP	3.91 ± 0.30 <sup>b</sup>	1.98 ± 0.15 <sup>b</sup>	1.31 ± 0.10 <sup>b</sup>
MPTP+ Ws	2.44 ± 0.19 <sup>c</sup>	1.31 ± 0.16 <sup>c</sup>	0.98 ± 0.07 <sup>c</sup>
Ws	1.42 ± 0.11 <sup>a</sup>	0.79 ± 0.06 <sup>a</sup>	0.69 ± 0.05 <sup>a</sup>

<sup>A</sup> = amount of enzyme required to inhibit 50% of NBT reduction, <sup>B</sup> =  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein. Values are expressed as means ± SD (n = 6). Values not sharing a common superscript letter differ significantly at  $p < 0.05$  (DMRT).

## DISCUSSION

In the first day or two, we observed a slight reduction in the body weight of the mice given MPTP (Group II) or MPTP + Ws, but this was non-significant relative to the controls. The mice of both groups soon recovered, as seen in other studies [20].

The ability to balance and walk on a rotating rod is widely used as a measure of motor skill and coordination [13]. MPTP-injected mice displayed a reduced mean hind limb stride length compared with their forelimbs, resembling the reduced stride length in PD gait [14]. In this study, the rotarod and hang test performances for the MPTP group were significantly worse than those for the control.

The substantia nigra is a small nucleus located in the ventral midbrain. Its primary function appears to be the control of motor function, and it forms a part of what is commonly known as the 'motor circuit'. The disruption of such

a system may result in movement disorders such as those seen in Parkinson's disease [21]. Treatment with *Withania somnifera* reversed the alterations in locomotor and muscle coordination in 6-hydroxy dopamine-induced parkinsonic rats via an unknown mechanism [22], which is corroborated by our findings.

Substantia nigra (SN) neurons are more vulnerable to oxidative stress due to the presence of high concentrations of iron and low contents of endogenous antioxidants relative to the other regions of the brain [23]. Increased levels of malondialdehyde (MDA) and lipid hydroperoxides [24, 25], which are markers of oxidative damage, were reported in the midbrains of PD patients. In the brain, MPTP is metabolized to its active toxin, MPP<sup>+</sup>, by the action of monoamine oxidase (MAO) [26]. This enzymatic conversion of MPTP to MPP<sup>+</sup> is shown to involve the generation of free radicals [27]. Rojas and Rios [28] found enhanced lipid peroxidation after MPP<sup>+</sup> administration to mice, a process dependent on the overproduction of free radicals. Similarly, the formation of superoxide anions in the presence of MPP<sup>+</sup> [29] results in increased activity of SOD in SN [30]. Acute treatment with MPTP caused a significant enhancement in the levels of TBARS and increased the specific activity of SOD and CAT in both the striatum and midbrain [12, 31].

*Withania somnifera* is also known as ashwagandha in Ayurveda, the ancient Hindu system of medicine. It has been in use for more than 2500 years and is reported to have anti-tumour [32], cardioprotective [33], antioxidant [34] and anti-alzheimeric [35] properties. It also has positive effects on the endocrine [36] and central nervous systems [37]. The ethanolic extract of *Withania somnifera* increases the striatum dopamine content and attenuates the effects of 6-hydroxy dopamine-induced parkinsonism in rats. In conclusion, this study provides evidence that ashwagandha possesses antioxidant properties, and indicates further study is necessary to establish its neuroprotective role.

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