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Original Article

Restorative Effect of Pioglitazone against MPTP Mediated Behavioural Deficits in Mice Model of Parkinson's Disease

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ABSTRACT

This study was designed to assess the protective effect of pioglitazone against behavioural changes associated with experimentally induced Parkinson's disease (PD) in mice model. PD was induced in male C57BL6/J mice by injecting five consecutive doses (25mg/kg/day ip) of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) hydrochloride at 24 h interval. The mice were then treated orally with pioglitazone (20mg/kg) once a day for 10 consecutive days after MPTP administration. Behavioural studies like open field, rota rod, hang grid, stride length and olfactory tests were carried out on 8th day of post MPTP injection and on 11th day of post pioglitazone treatment to assess PD associated behavioural deficits and the effect of pioglitazone in improving the behavioral manifestations. Data obtained from different behavioral tests in this study indicated that MPTP treated mice showed persistent behavioral deficits in the balance and motor coordination skills on the challenging rotarod, hanging grid, stride length gait consistency pattern, olfactory function and spontaneous locomotor behaviours in the open field test. Grippingly, chronic post treatments with pioglitazone caused significant improvement in MPTP-induced behavioral deficits and were successfully diminished.

Keywords: *Substantia nigra pars compacta* (SNpc), C57BL6/J mice, Grid test, Open field, Rotarod, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP).

INTRODUCTION

Parkinson's disease (PD) is characterized by the progressive degeneration of numerous neurons inhabiting different regions of the brainstem, most remarkably of dopaminergic neurons located in the *substantia nigra pars compacta* (SNpc)[1] and the deficit of dopamine is correlated with a complex onset of motor symptoms[2].

The disease is characterized by bradykinesia, rigidity, postural instability, orofacial dyskinesia, muscular stiffness and tremor. Other non motor complications include sleep disorders and cognitive impairment[3], depression[4], mood fluctuations[5], psychosis and dementia[6] etc. Secondary manifestations like defective posture and gait, mask like face and sialorrhoea, difficulty in breathing, chest infection/embolism may also develop.

The toxin, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is known to cause neurotoxic effects in several species via degeneration of dopaminergic neurons and this is initiated by the active toxic metabolite of MPTP, 1-methyl-4-phenyl-pyridinium ion (MPP^+) formed by oxidation of monoamine oxidase B (MAO-B). Thereafter, MPP^+ released in the extracellular space is taken up by the high affinity dopamine transporters and subsequently accumulated within the mitochondria of the nigrostriatal dopaminergic cells[7,8] leading to neuronal cell death.Thereby striatal content of MPP^+ is linearly and positively correlated with the magnitude of dopaminergic damage[9].

In general, nonhuman primates and C57BL/6J mice, are the widely used MPTP models for Parkinson's disease[10-13] and however large frequent doses of MPTP injections are required for depleting striatal dopamine contents in mice[14].

In addition, the PPARγ agonist pioglitazone, a centrally penetrant anti-diabetic thiazolidinedione, has been shown to have anti-inflammatory and neuro-protective effects in animal models of Parkinson's[15] and Alzheimer's disease[16]. Previous investigations have also assessed the positive effects of pioglitazone pre-treatment (20mg/kg/day) in MPTP mice model of Parkinson's disease[15,17,18]. In the present study we investigated the effect of pioglitazone post-treatment p.o (20mg/kg/day) against motor deficit and dopamine depletion, in C57BL/6J mice induced with Parkinson's disease by administering five consecutive doses of MPTP injections (25mg/kg/day, i.p.). Furthermore, the behavioural studies were examined in mice after disease induction and pioglitazone treatment.

MATERIALS AND METHODS

Chemicals

MPTP was obtained from *Sigma Aldrich, St Quentin, France.*

Drug

Pioglitazone hydrochloride (Obtained as a gift sample from *Bonn Schterin Biosciences*, Puducherry).

Maintenance of animals

Male C57BL6/J mice of body weight 35-40g supplied by Ragavendra suppliers, Bangalore were used in this study. Animals were allowed to acclimatize for atleast 1 week before the start of the study and were fed on the standard pellet diet (NPD) for acclimatization. Water was given *ad libitum*. The animals were housed in plastic cages under controlled condition of 12h light / 12h dark cycles, 50% humidity and at 30 ± 2°C. The animals used in the present study were maintained in accordance with the

guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India. The present work is approved by the Institutional Animal Ethical Committee (IAEC), Pondicherry University (PU/IAEC/10/32).

Animals and treatment

C57BL6/J mice in different groups received five consecutive intra-peritoneal injections of MPTP-HCl (25 mg/kg/day in saline; *Sigma Aldrich, St Quentin, France*) for a period of 5days.Control mice received saline only. After the mice were induced Parkinson's disease, behavioral tests were continued to assess the level of disease progression. Based on the behavioral test results, the animals were left for 7 days after disease induction for the onset of PD. After 7 days they were treated orally with 20 mg/kg bodyweight of *Pioglitazone hydrochloride* (Obtained as a gift sample from *Bonn Schterin Biosciences*, Puducherry) for a period of 2 weeks.

The effects of pioglitazone post-treatment on MPTP-induced behavioural impairments

All the behavioral tests were carried out on 8th day of post MPTP injection and on 11th day of post pioglitazone treatment.

Motor integration tests were assessed by different methods

Rota rod test

Motor co-ordination was measured using an automated rotarod. For this procedure, the mice were pre-trained on the rotarod and the animals were exposed to 3 trials on rotating rod at 15 rpm with a time interval of 5 minutes and cutoff time of 180 seconds[19]. Mice were continuously allowed to walk forward and their ability to maintain balance on the accelerating rotating rod was observed by recording their fall off time in seconds. The average of the retention time on the rod was calculated.

Grid hang test

Neuromuscular strength was determined using the grid hang test[20]. One advantage of the grid test is that it takes into account the general locomotor activity of the mouse and the neuromuscular strength was determined in the grid hang test. The mice lifted by their tails, slowly placed on a meshed sieve, a horizontal grid and supported until they grab the grid with their fore and hind limbs. The grid then inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface to discourage falling, but not leading to injury in case of a fall. The 12 mesh sieve equipped with a 3 inch wall was taken as a grid to prevent the animals from traverse on the upper side of the grid. The time required for the animals to stay on the grid for a period of 5 minutes and the maximum hanging time was recorded.

The % of hanging time is calculated by the formula: - (Maximum hanging time X 100) /300 seconds.

Gait analysis by stride length test

Gait disturbances, including the width and length of each paw print are associated with various conditions including spinal cord injury, neuropathic pain, muscular and neurological diseases. To measure gait, animals were trained to walk through a narrow alley leading into their home cage. Once trained, paper was placed along the alley floor (a white paper was placed on a narrow wooden plank) and each animal's forelimbs and hind limbs were brushed with nontoxic color (red and blue). Animals were then placed at the beginning of the alley. As they walked into their home cage, they left their paw prints on the paper underneath[21] and the stride lengths were determined by

measuring the distance between the paw prints.

Olfactory test

To measure general olfactory function, the buried pellet test can be used. The test relies on the mouse locating a hidden object, usually a food pellet, by odour[22-24]. The amount of time taken by the food-restricted animal to find and uncover the food pellet is measured. Alternately, dry fish is used in the present study instead of food pellet.

Open field test

Open field consists of a rectangular box (40x50x63cm) whose floor is divided into 20 small squares. The test is made by placing the mice in the right corner of the open field and was allowed to walk without restraint inside the area for 5 min. The immobility duration (time of total absence of paw movements), rearing frequency (partial or total elevation onto hind limbs), locomotion frequency (number of squares crossed), and parameters like frequency of scratching and defecation were determined, using hand operated counters[25].

RESULTS

Rota rod test

Figure 1 depicts the results of the rotarod test. MPTP-treated mice on 8th day exhibited significant reduction ($p<0.05$) in the retention time on the rod indicating a loss of motor coordination. On the other hand behavioral study in MPTP treated mice observed on the 11th day of pioglitazone post treatment showed significant improvement ($p<0.05$) in the retention time as compared to disease induced mice.

Grid hang test

Results obtained in the Grid hang Test are shown in Figure 2 which depicts the hanging time taken by MPTP-treated mice and those mice treated with pioglitazone. The hanging time taken by MPTP-treated mice was significantly reduced as compared to control animals ($p<0.05$). Post treatment with pioglitazone (20 mg/kg) caused significant improvement in the performance ($p<0.05$) and showed a better hanging time when compared to the hanging time of MPTP-treated mice.

Stride length test

The gait disturbances observed in mice on the 8th day of post MPTP injection and on 11th day of post pioglitazone treatment as analyzed by the stride length test are presented in Figure 3. Persistence of abnormal gait with a decrease in the stride length was noted in MPTP treated mice when compared to the control mice on the 8th day of post MPTP injection. On the other hand, observation on 11th day in post pioglitazone treated mice had shown better readouts of gait patterns. Increase in stride length measurement as compared to MPTP treated mice by significantly reversing the stepping patterns and swing duration of the paws was seen with pioglitazone treated mice ($p< 0.05$).

Olfactory test

The results of the olfactory test in pioglitazone treated MPTP mice models are shown in Figure 4. The general olfactory function was detected in mice by allowing it to locate a dry fish by odour and the total time spent in exploring the dry fish was observed with a cut off time of 5 minutes. However, observations made on the 8th day of post MPTP administration indicated prolonged time of reaching the dry fish by sniffing its odour as compared to the control mice ($p<0.05$). Whereas, observation on 11th day of pioglitazone post treatment in MPTP mice showed that lesser time was taken for locating the dry fish through reversing its the olfactory deficit.

Open field test

Figure 5 and 6 depicts the locomotory behaviours of mice in the open field test recorded on the 8 day of post MPTP administration and on 11th day of pioglitazone post treatment. Observations were recorded for a period of 5minutes. It was observed that the MPTP challenged mice displayed significantly less ambulatory activity and increased time of immobility than that of the control mice whereas the pioglitazone treated mice showed increased ambulatory activity.

DISCUSSION

Various mammalian species, including sheep, dogs, guinea pigs, cats, mice, rats, and monkeys, have been treated with MPTP to induce PD[26,27]. MPTP in mice primarily damages the nigrostriatal dopaminergic pathway[28,29] and this specificity and reproducibility in eliciting neurotoxic effects to the nigrostriatal system serves as a strength of this model. Also, reports indicating MPTP mediated motor deficits in monkeys and mice confirm the connection with the nigrostriatal damage[30].

Many behavioral tests have been used in MPTP-induced PD mouse model and selection of these tests depends on their robustness and sensitivity to detect functional changes after challenge by a neurotoxin. The widely employed rotarod test is a feasible mean to analyze coordination disorders in PD mouse model[19]. The grid test serve as a sensitive method to assess the striatal DA level in MPTP-treated mice, but are heavily dependent on the skilled manipulation of fore-limbs of the animal being studied [20]. The open field test is commonly used for evaluating motor deficits like locomotor activity, period of immobility in mice following MPTP insult[31,32]. In the same way, olfactory disturbances are the non-motor symptoms observed in PD. Behavioural testing of olfaction can facilitate an earlier detection of PD, since impaired olfaction has been positively correlated with an increased risk of developing the disease[33] where the olfactory function in mice were generally detected by allowing the mice to locate the hidden food by sniffing its odour[22-24]. In addition, the gait analysis technique of measuring and comparing the variability of stride length and stepping pattern in mice can also be used in rodent models of Parkinson's disease[34,35]. Pioglitazone, as a PPARγ agonist and an effective insulin sensitizer, was reported for its beneficial effects in number of neuro-degenerative disease models [36,37].

The present study examined the protective effect of *pioglitazone hydrochloride* in MPTP mice model of Parkinson's disease. This study also provides new evidence on the action of the PPARγ agonist pioglitazone post induction of PD after the administration of the neurotoxin, MPTP to C57BL/6J mice. In the present study, mice treated with 5 consecutive injections of MPTP (25mg/kg/day ip) to induce PD showed persistent increase in chronic behavioral (motor and non motor) deficits in association with the development of Parkinson's Disease. Through the behavioural studies performed on the 8th day of MPTP post-treatment, we visualized significant deficits in (1) the balance and motor coordination skills on the challenging rotarod and (2) hanging grid; (3) gait consistency pattern on stride length; (4) olfactory function;(5) the spontaneous locomotor behaviours in the open field, etc.

C57BL/6J mice treated with MPTP spent less time on the rotating rod indicating a loss of motor coordination while pioglitazone treated mice showed improved rotarod performance. The neuromuscular strength as assessed by Hang test also suggested a reduction in the hanging time following MPTP treatment but pioglitazone post treatment enhanced the neuromuscular strength significantly by enhancing the

hanging periods. Administration of MPTP also reduced the spontaneous locomotor activity with an increase in freeze time while pioglitazone post treatment revived the animals from hypo locomotion and increased period of immobility. Similarly significantly decreased stride length shown by MPTP treated group was found to be improved by pioglitazone treatment. Pioglitazone treatment also caused improved the olfactory senses of mice and enabled them to locate the food in shorter time as compared to MPTP treated mice.

The positive effect of chronic post treatment with pioglitazone (20mg/kg/day p.o) for 10 days in the behavioural pattern of MPTP-treated mice was attention-grabbing. Noticeably, improvement in the behavior of animals observed after the 11^{th} day of post pioglitazone treatment. Hence in the present study, MPTP-induced behavioral deficits that lasted for nearly 4 weeks were successfully diminished by pioglitazone post treatment.

CONCLUSION

Our current understanding of Parkinson's disease has greatly benefitted from the behavioural studies in MPTP animal model. Our present study offers further evidences on the behavioural changes associated with progression of Parkinson's disease in MPTP mice model and brings out positive effects of pioglitazone against behavioural deficits induced by the neurotoxin MPTP.

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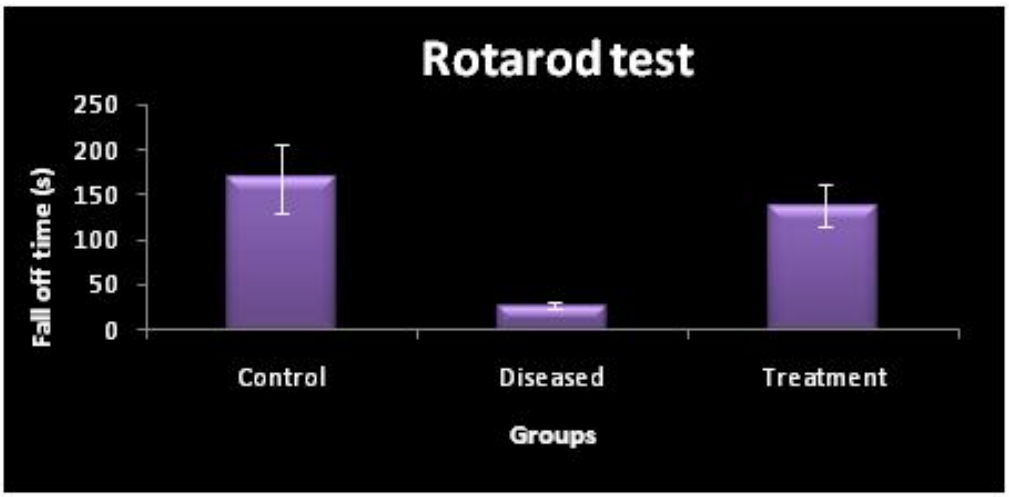

Figure 1. Variation in the performance measured as retention time at 15 rpm in MPTP mice treated with *Pioglitazone hydrochloride*

Values are expressed as mean± SD (n= 4).
*-represents statistical significance at 0.05 level ($p<0.05$)
One Way ANOVA followed by Tukey's HSD.

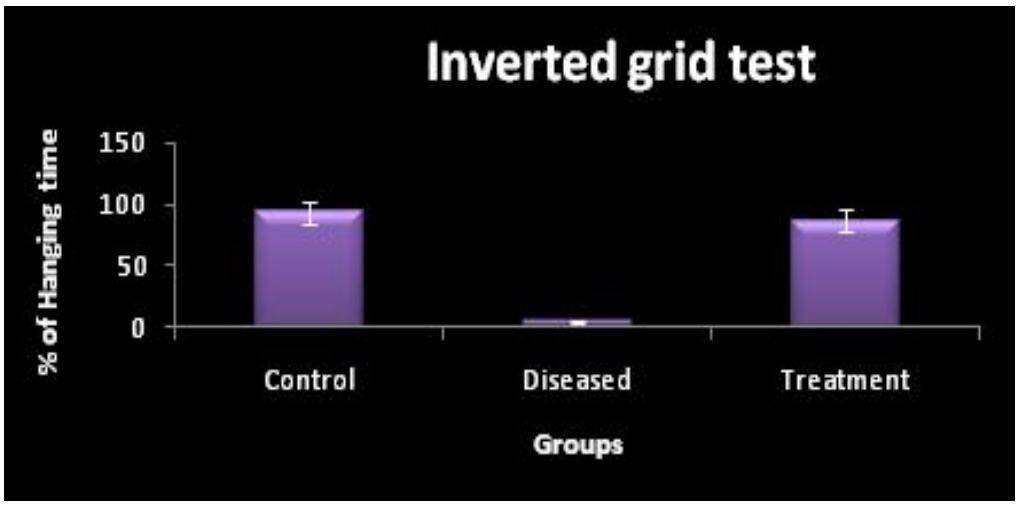

Figure 2. Hang test in MPTP- mice treated with *Pioglitazone hydrochloride*

Values are expressed as mean± SD (n= 4).
*-represents statistical significance at 0.05 level ($p<0.05$)
One Way ANOVA followed by Tukey's HSD.

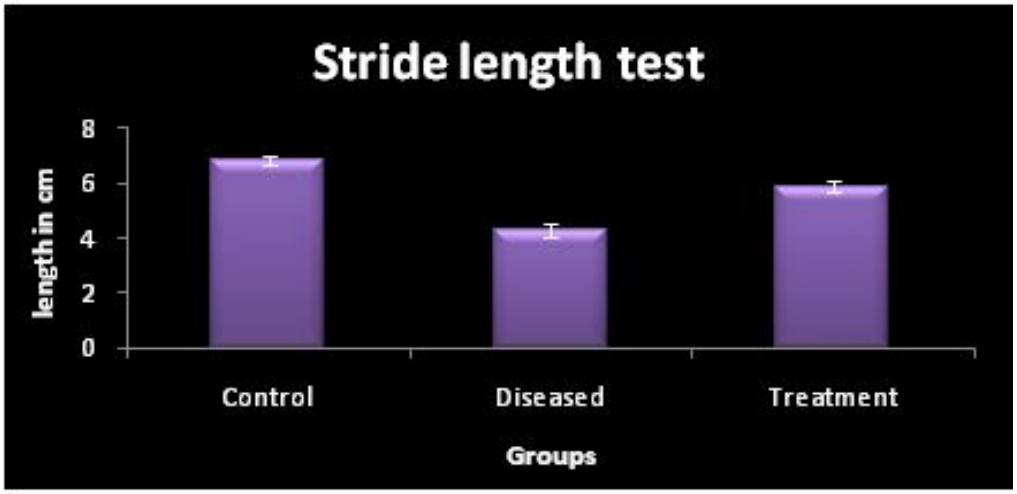

Figure 3. Stride length test in MPTP- mice treated with *Pioglitazone hydrochloride*

Values are expressed as mean± SD (n= 4).
*-represents statistical significance at 0.05 level ($p<0.05$)
One Way ANOVA followed by Tukey's HSD.

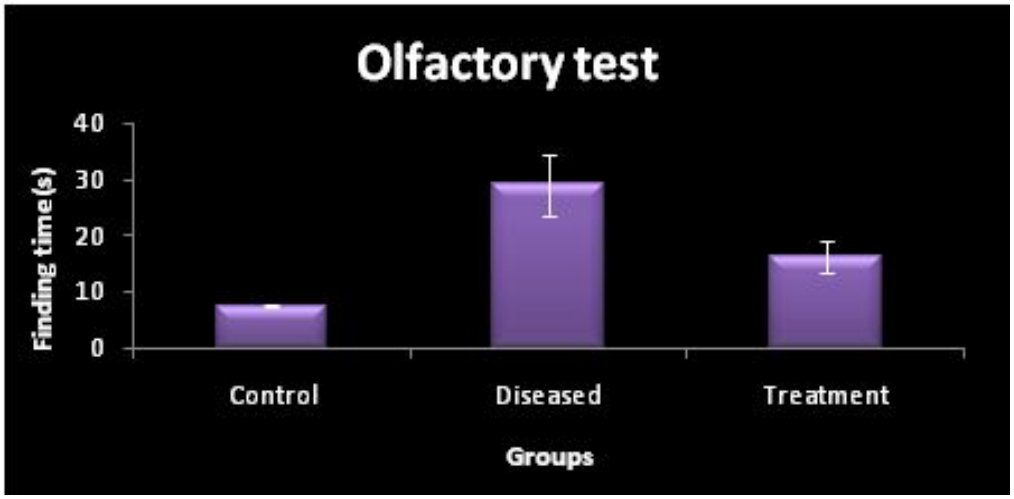

Figure 4. Olfactory test in MPTP- mice treated with *Pioglitazone hydrochloride*

Values are expressed as mean± SD (n= 4).
*-represents statistical significance at 0.05 level ($p<0.05$)
One Way ANOVA followed by Tukey's HSD.

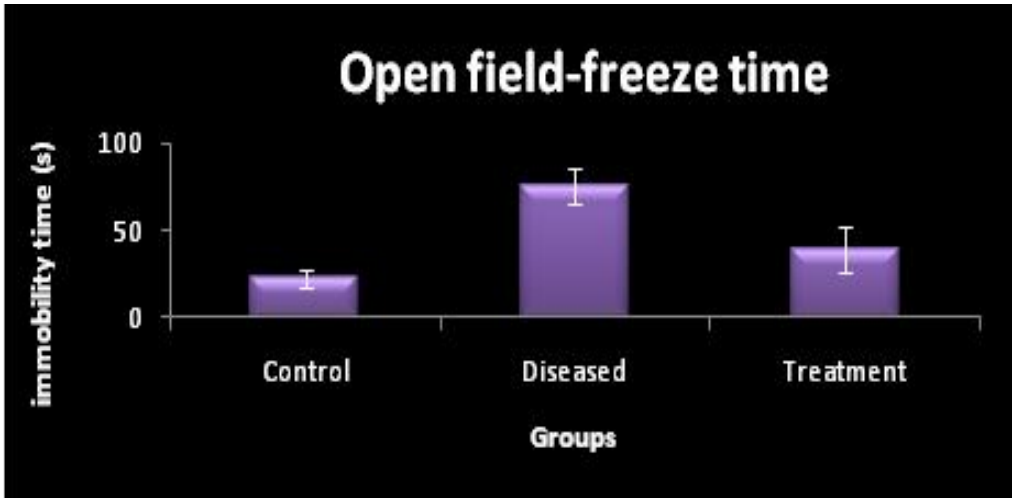

Figure 5. Open field test determining immobility time in MPTP-mice treated with *Pioglitazone hydrochloride*

Values are expressed as mean± SD (n= 4).
*-represents statistical significance at 0.05 level ($p<0.05$)
One Way ANOVA followed by Tukey's HSD.