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The behavioral performance tests of *Mucuna pruriens* gold nanoparticles in the 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine treated mouse model of Parkinsonism

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ABSTRACT

Objective: The present investigation is aimed to carry out the behavioural changes of the *Mucuna pruriens* extract (MPE) and *Mucuna pruriens* Goldnanoparticles (MPGNPs) against MPTP-induced neurotoxicity. **Methods:** MPGNPs prepared from the methanolic extract of *Mucuna pruriens* seeds using Chloroauricchloride ($HAuCl_4$). The seed powder has been found to show the anti-parkinsonian effects. Mice were induced with 10 mg/kg of MPTP, four injections i.p., at 1 h intervals within 24 h. MPE was administered at the dose 200 mg/kg (i.p) and MPGNPs was administered at different doses of 500 μg/kg, 5, 10 and 20 mg/kg (i.p) in different groups once a day for seven days and the dose on the first day was given 30 min prior to first MPTP injection. **Results:** The behavioural changes were studied using the rotarod test, hang test and narrow beam test. MPE and MPGNPs significantly ($P<0.05$) improved the behavioural activities. **Conclusions:** MPGNPs possesses significant behavioural activity than MPE against MPTP induced neurotoxicity.

1. Introduction

Parkinson's disease (PD) is a progressive, disabling neurodegenerative disorder of unknown cause, characterized by bradykinesia and at least 1 of the following symptoms resting tremor, muscle rigidity, and postural instability [1,2]. It is the second most common neurodegenerative disorder, after Alzheimer's disease [3]. It is due to Damage or loss of dopaminergic neurons in this brain region results in the depletion of dopamine from terminals in the striatum/nucleus caudatus putamen involved in coordinating smooth movement. 1-methyl-4phenyl-1, 2, 3, 6- tetra-hydropyridine (MPTP) is a potent neurotoxin that produces nigral dopaminergic neuronal damage in humans, primates and rodents [4]. *Mucuna pruriens* Linn (MPL) is a popular Indian medicinal plant, which has long been used in Ayurvedic system of medicine for diseases including Parkinsonism [5]. The seed powder has been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA. As L-DOPA is the precursor of dopamine, it crosses the barrier and gets converted into dopamine resuming the neurotransmission [6]. Due to the numerous protective barriers surrounding the CNS, there is an urgent need for effective treatment for patients living with PD. There is a need to search for alternative medicine, with neuroprotective effects, without side effects, for the management of PD. Therefore nanotechnology may provide a possible solution for the treatment of AD and PD by affording targeted drug delivery and enhancing the bioavailability and/or efficacy of various drugs and other bioactive agents used in Neurodegenerative Diseases [7]. In recent years the research and applications in the area of nanoscience and nanotechnology are well developed [8]. Gold nanoparticles have found use in biomedicinal applications particularly diagnostic and drug delivery [9]. There are numerous methods available using various approaches including chemical, physical, and biological protocols for the synthesis of nanoparticles. The physical and chemical methods are very expensive and yield toxic substance. The need for eco-friendly non-toxic methods for nanoparticles synthesis is developing interest in biological approaches which are free from the use of toxic chemicals as byproducts [10-12]. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles [13]. So, the present study has been designed to evaluate the

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behavioural changes of the *Mucuna pruriens* extract (MPE) and *Mucuna pruriens* Goldnanoparticles (MPGNPs) against MPTP-induced neurotoxicity.

2. Materials and Methods

2.1. Experimental Animals

Male Swiss albino mice, Mus musculus, weighing approximately 25 to 30 g, were acclimatized at room temperature (28 ± 3 ℃) and relative humidity (55%) in a 12-hour light/dark cycle in a room under hygienic condition, were used in the present experiments. The animals reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University were used for the experiment, and were fed on a standard balanced diet (Hindustan Lever, Bangalore) and provided with water ad libitum. All studies were conducted in accordance with the National Institute of Health Guide.

2.2. Chemicals

MPTP was obtained from Sigma-Aldrich Co. (St. Louis, USA). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) was obtained from Loba Chem. Loba chemie pvt Ltd, Mumbai, India.

2.3. Plant Material

M. pruriens seeds were purchased from the local herbal market, Chidambaram, Tamil Nadu, India. The dried seeds were made into fine powder with an auto-mix blendor and were kept separately in an airtight container until use. The methanolic extract prepared using Soxhlet was concentrated by rotary evaporator at 40℃ and stored in a cool place.

2.4. Synthesis of Gold Nanoparticles

100 ml of 1mM solution of chloroauric acid was kept in a 250 ml Erlenmeyer flask. 100 ml of *M. pruriens* supernatant was added to the chloroauric acid solution. 95% of bioreduction of $AuCl_4^-$ ions occurred within 10 min. The yellow colored solution turned into purple red slowly indicating the formation of *M. pruriens* gold nanoparticles (MPGNPs) [14].

2.5. Experimental design

The animals were randomly divided into 6 groups, each of eight animals. Group I served as vehicle control (Saline 10% + DMSO 4 ml/kg BW; i.p), Group II administered with 400 mg/kg MPE (i.p.,), Group III received 20 mg/kg MPGNPs (i.p.,), Group IV received 10 mg/kg MPTP (i.p.,) at 1 h intervals with total dose of 40 mg/kg as previously described [15], Group V received 200 mg/kg MPE (i.p.,), Group VI, VII, VIII, IX received 500 μg/kg, 5, 10 and 20 mg/kg MPGNPs (i.p.,) respectively. The treatment was given on the initial day, 30 min. prior to first injection of MPTP and once a day for another six days of the experimental period.

2.6.Motor integration tests were assessed by different methods

2.6.1. Rotarod test

Motor co-ordination was measured on 3rd and 7th day using an automated rotarod (Amni, Rotar Instrumentation, Columbus, OH, USA). Animals were exposed to ten trials on rotating rod at various rpm such as 5, 10, 15 with 5 min intervals and the cutoff time was 180 sec. [16]. The rotor was divided into five compartments, which could allow five mice at a time. The average of the retention time on the rod was calculated.

2.6.2. Narrow beam maze (NBM) test

Motor co-ordintation and balance were assessed on 3rd and 7th day by measuring the ability of the mice to traverse a narrow beam to reach a goal box [17].

2.6.3. Hang test

Neuromuscular strength was determined on 3rd and 7th day in the grid hang test [18].

2.7. Statistical Analysis

One way analysis of variance followed by Duncan's Multiple Range Test was employed for the analysis of behavioural variances. The results were considered statistically significant if the p value is less than 0.05.

3. Results

3.1. Rotarod test

Table 1 depicts the results of the rotarod test. MPTP-induced mice on 3rd and 7th day exhibited significant decrease in the retention time on the rod indicating a loss of motor coordination. Treatment with MPE and MPGNPs improved the retention time as compared to MPTP- mice on both 3rd and 7th days. 10 mg and 20 mg of MPGNPs treated mice showed better retention time than 500 μg and 5 mg. The MPGNPs treated animals showed better retention time than MPE.

3.2. Narrow beam test

Table 2 depicts the results of the narrow beam walk and Hang test in MPTP-mice on treatment with MPE and MPGNPs on 3rd and 7th days. The time taken to cross between the starting point and goal box by MPTP-mice was significantly increased as compared to control. The hanging time taken by MPTP-mice was significantly reduced as compared to control. Treatment with MPE and MPGNPs showed significantly reverse the crossing time and hanging time when compared to MPTP- mice 3rd and 7th day. The 10 mg and 20 mg of MPGNPs treated mice showed similar and better crossing time and hanging time than 500 μg, 5 mg/kg. Administration of MPGNPs treated animals showed better crossing time and hanging time than MPE on both 3rd and 7th day.

4. Discussion

The administration of the neurotoxin MPTP, reported to induce clinical symptoms of PD in both humans and experimental animals by selectively damaging the dopaminergic nigrostriatal system, resulting in the loss of dopaminergic neurons in the SN and a reduction of dopamine in the ST [19]. The present study, clearly showed

Table 1

Variation in the rotarod performance measured as retention time at 5, 10,15 rpm in MPTP- mice treated with *Mucuna pruriens* extract (MPE) and *Mucuna pruriens* gold nanoparticles (MPGNPs) on 3rd and 7th days.

Groups	5 rpm		10rpm		15 rpm	
	3 rd day(s)	7th day(s)	3 rd day(s)	7th day(s)	3 rd day(s)	7th day(s)
Control	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]
MPE (200 mg/kg BW)	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]
MPGNPs (20 mg/kg BW)	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]	180.00±0.00[a]
MPTP (40 mg/kg BW)	75.02±1.79[b]	39.50±1.34[b]	64.58±1.24[b]	30.86±1.00[b]	59.55±1.17[b]	25.84±1.91[b]
MPTP + MPE (200 mg/kg BW)	116.31±2.71[c]	130.09±2.94[c]	108.98±1.99[c]	122.23±2.16[c]	97.19±4.58[c]	114.69±3.09[c]
MPTP + MPGNPs (500 μg/kg BW)	81.27±1.56[d]	85.67±2.59[d]	70.22±3.21[d]	74.87±1.37[d]	67.18±3.17[d]	70.09±1.96[d]
MPTP + MPGNPs (5 mg/kg BW)	110.92±4.67[e]	112.44±3.56[e]	97.63±2.60[e]	106.08±3.69[e]	92.91±2.66[e]	102.28±1.95[e]
MPTP + MPGNPs (10 mg/kg BW)	125.64±4.34[f]	140.90±3.33[f]	119.52±3.46[f]	135.90±2.02[f]	108.99±3.09[f]	127.57±2.95[f]
MPTP + MPGNPs (20 mg/kg BW)	124.95 ±5.93[f]	140.52±4.82[f]	118.75±5.24[f]	134.64±4.98[f]	106.74±2.09[f]	126.03±3.18[f]

Values are expressed as means±SD for eight animals in each group.
Values not sharing a common superscript differ (a, b, c..) significantly at $P<0.05$ DMRT.

Table 2

Narrow beam and Hang test in MPTP- mice treated with MPE and MPGNPs on 3rd and 7th days.

Groups	Narrow beam		Hang test	
	3 rd day(s)	7th day(s)	3 rd day(s)	7th day(s)
Control	3.00±0.03[a]	3.02±0.01[a]	29.89±0.13[a]	29.95±0.19[a]
MPE (200 mg/kg BW)	2.97±0.05[a]	2.99±0.06[a]	29.86±0.10[a]	29.93±0.14[a]
MPGNPs (20 mg/kg BW)	3.01±0.02[a]	2.97±0.03[a]	29.82±0.13[a]	29.91±0.05[a]
MPTP (40 mg/kg BW)	26.22±1.18[b]	39.13±1.11[b]	8.59±0.73[b]	3.55±0.31[b]
MPTP + MPE (200 mg/kg BW)	11.00±0.51[c]	9.00±0.59[c]	17.71±0.51[c]	22.08±0.97[c]
MPTP + MPGNPs (500 μg/kg BW)	20.12±1.89[d]	18.19±1.49[d]	12.49±0.95[d]	14.33±1.15[d]
MPTP + MPGNPs (5 mg/kg BW)	13.00±0.67[e]	12.03±0.73[e]	15.58±1.02[d,c]	18.77±1.37[e]
MPTP + MPGNPs (10 mg/kg BW)	8.49±0.45[f]	6.22±0.55[f]	23.85±2.01[e]	26.99±1.48[f]
MPTP + MPGNPs (20 mg/kg BW)	8.69±0.52[f]	6.40±0.47[f]	20.90±1.89[e]	25.49±1.99[f]

Values are expressed as means±SD for eight animals in each group.
Values not sharing a common superscript (a, b, c...,) differ significantly at $P<0.05$ DMRT.

that mice induced with the dopaminergic neurotoxin MPTP could develop a variety of behavioural deficits such as loss of muscular coordination, loss of memory, loss of neuromuscular strength, loss of balance and mobility time. The dopamine is the main neurotransmitter involved in normal motor function, alteration in the brain dopamine levels can produce modification in the motor function [20].

The rotarod test has been extensively used to measure the motor co-ordination in experimental animals. Narrow beam test is required to measure the locomotor ability in mice. Neuromuscular strength was assessed by hang test. In the present study, the MPTP regimen shows impaired rotarod performance, altered locomotor performance, which took more time to cross the beam, significant reduction in the hang time. The treatment of MPE and MPGNPs in MPTP-induced mice significantly reverses the retention time in rotarod, crossing time in narrow beam and the hang time in hang test. It was reported that the MPE was significantly improved the Locomotor activity [21].

M. pruriens is reported to contain L-DOPA as one of its constituents. L-DOPA is best known to the world as a treatment for PD, a neurological disorder. It is the precursor for the neurotransmitter DA [22]. It has been reported that, the *M. pruriens* seeds are used for treatment of the nervous system, including Parkinson's disease and potentiation of growth hormone [23]. In the present study, the behavioural impairments in motor coordination beam walking and hanging test improved on treatment with MPE and MPGNPs that might be due to presence of L-DOPA in *M. pruriens*. Manivasagam *et al*., reported that the synergistic neuroprotective effect of WsRp and MpSp combination improves the neurochemical levels, antioxidant status and behavior patterns significantly. These results suggested that the synergistic antiparkinsonic effects of WsRp and MpSp and these findings also provided a therapeutic basis for the clinical application of this drug pair [24]. In the present study, treatment with MPE and MPGNPs prevented the motor impairments and improved the behavioural activities. The animals which were administered 10 mg/kg MPGNPs had a significant improvement than other doses. The improved motor performance also correlated with improved dopamine elevation on treatment of MPE and MPGNPs.

Biosynthesis of nanoparticles employing microorganisms or plants can potentially eliminate the toxicity problem by making the nanoparticles more biocompatible [25]. In a previous report, GNPs are able to cross the blood-brain barrier and accumulate in the neural tissue. Importantly, no evidence of toxicity was observed in any of the diverse studies performed, including survival, behavior, animal weight, organ morphology, blood biochemistry and tissue histology [26]. Nanoparticles are able to penetrate the blood brain barrier of in vitro and in vivo models disrupting the temporally the barrier and allowing the incorporation the therapeutic agents into the brain [27]. Nanoparticles are currently being used to refine the discovery of biomarkers and molecular diagnostics, which could be applicable to the management of neurodegenerative and neurological diseases. Therefore, new therapies for these hard to

treat brain diseases are needed urgently alongside brain malfunctions such as Alzheimer's, Parkinson's and more [28, 29]. In our study, the MPGNPs significantly improved the behaviour performance. So, it is concluded that MPE and MPGNPs can exert a significant neuroprotective effect.

Conflict of interest

We declare that we have no conflict of interest

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