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International Journal of Current Research
Vol. 5, Issue, 10, pp. 3251-3257, October, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF *Ginkgo biloba* EXTRACTS AND *Ginkgo biloba* GOLD NANOPARTICLES ON 1-METHYL-4-PHENYL-1,2,3,6-TETRA HYDRO PYRIDINE INDUCED BEHAVIORAL DEFICITS IN MICE MODEL OF PARKINSON'S DISEASE

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ARTICLE INFO

Article History:
Received 28th August, 2013
Received in revised form
17th September, 2013
Accepted 29th September, 2013
Published online 23rd October, 2013

Key words:
Nanotechnology, Behavioural test,
MPTP, *Ginkgo biloba.*

ABSTRACT

The present study aimed to evaluate the effect of *Ginkgo biloba* extract (GBE) and *Ginkgo biloba* gold nano particles (GBGNPs) on MPTP induced behavioral change in Parkinson's disease mouse, investigated through the following behavioral test such as rotarod performance hang test, narrow beam walking, akinesia and catalepsy. The results indicate a significant variation in behavioral patterns of control and experiment groups. *G.biloba* may be useful for the management of neuropathic pain. When compare to GBE the GBGNPs showed better improvement in all behaviour tests.

INTRODUCTION

Parkinson's Disease (PD) may be associated with several signs and symptoms of Autonomous Nervous System (ANS) impairment, which have been already referred since the original description of the disease by James Parkinson in 1817 (Parkinson, 1817; Giza *et al* 2012). PD is a neurodegenerative disorder, characterized by resting tremor, rigidity or stiffness, bradykinesia, and postural instability. Although PD was described almost two centuries ago, its etiology remains unclear (Schapira and Jenner, 2011; Wirdefeldt *et al.,* 2011; Sara Mínguez-Míngueza *et al.,* 2013). There are several models are available to study the PD, mouse models using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are among the most widely used. MPTP mouse models have shed light on the pathophysiology, as well as some of the causes of the disease. More importantly, they have provided investigators with model platforms for testing symptomatic and neuroprotective drugs (Gloria Meredith and David Rademacher, 2011). Systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetra hydro pyridine (MPTP) to mice is an established experimental model of idiopathic Parkinson's Disease (PD), as this toxin produces marked depletion of striatal dopamine (DA), its metabolites and terminals, and destruction of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc) (Jackson-Lewis *et al.,* 1995; Quinn *et al.,* 2007). The healing ability of *G. biloba* has been reported for thousands of years. It is one of the most important medicinal plant in the world, extensively used by many scientists and medical professionals to treat many problems related with aging, such as poor circulation, mental confusion and memory loss (Gertz and Kiefer 2004; Kamilla Blecharz-Klin *et al*., 2009). The most important constituents of the standardized extracts of dried leaves of *G. biloba* are flavone glycosides (quercetin, kaempferol, isorhamnetin) and terpene lactones (ginkgolides and bilobalide) (Mahadevan and Park 2008, Xie *et al.*, 2008). Drug delivery to the brain still remains highly challenging for the treatment of Alzheimer Disease (AD) and PD. The development of new practical treatment modalities for the treatment of neurodegenerative disorders is currently a highly active area of research. The application of technological advances in neurological research is expected to have a major impact leading to the development of newer therapeutic modalities (Girish *et al*., 2009). Nanotechnology could provide devices to limit and reverse neuropathological disease states, to support and promote functional regeneration of damaged neurons, to provide neuroprotection and to facilitate the delivery of drugs and small molecules across the blood brain barrier (Arulkumar and Sabesan, 2011). Based on the available literature, *G. biloba* gold nanoparticles were prepared by using *G. biloba* leaf extract. Various behavioral test such as rotarod performance (Rozas *et al.,* 1998), hang test, narrow beam walking (Anandhan *et al.,* 2012), akinesia and catalepsy (Haobam *et al.,* 2005) were used to analyse various aspects of motor functions. These methods are reported to be sensitive enough to detect functional impairments in MPTP-administered PD mouse models and to

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quantify the potential efficacy of treatments designed to restore dopaminergic function. These behavioral tests were largely designed to assess the innate motor skills/abilities of animals that are dopamine dependent, in order to relate the changes observed to the motor deficits seen in PD patients. The degree of dopamine loss, the timing and dose of the toxin injections, the time between injections and the behavioral testing and genetic manipulations will all impact the results of the behavioral study. Therefore, the present study aimed to evaluate the effect of GBE and GBGNPs against MPTP induced behavioral deficits in mouse model of PD by performing rotarod test, hang test, narrow beam walking test, stepping test (to measure akinesia and catalepsy).

RESULTS

Rotarod test

Tables 1-5 show the rotarod performance by MPTP-mice at various rpm (5, 10, 15, 20 and 25) on treatment with *G. biloba* leaf extract on 3rd and 7th days. All mice were trained on the rotating rod at a speed of 5, 10, 15, 20 and 25 rpm for 180 s. MPTP-induced mice on 3rd and 7th day exhibited significant decrease in the retention time on the rod when compare to control animals indicating a loss of motor coordination. Treatment with GBE improved the retention time as compared to MPTP- induced mice on both 3rd and 7th days.

Table 1. Variation in the rotarod performance measured as retention time at 5 rpm in MPTP- mice treated with *Ginkgo biloba* extract (GBE) on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBE (100 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	71.05 ± 1.25[b]	36.20 ± 1.32[b]
MPTP + GBE (25 mg/kg BW)	52.48 ± 3.35[c]	87.15 ± 1.80[c]
MPTP + GBE (50 mg/kg BW)	74.12 ± 3.95[d]	105.61 ± 5.41[d]
MPTP + GBE (100 mg/kg BW)	93.42 ± 1.58[e]	120.03 ± 3.34[e]

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.

Table 2. Variation in the rotarod performance measured as retention time at 10 rpm in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBE (100 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (20 mg/kg BW)	62.55±1.35[b]	25.82±1.45[b]
MPTP + GBE (25 mg/kg BW)	46.66±2.45[c]	75.04±2.52[c]
MPTP + GBE (50 mg/kg BW)	65.04±3.26[d]	90.06±2.21[d]
MPTP + GBE (100 mg/kg BW)	88.04±2.28[e]	112.34±1.87[e]

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.

Table 3. Variation in the rotarod performance measured as retention time at 15 rpm in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBE (100 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	58.53±1.35[b]	23.82±1.90[b]
MPTP + GBE (25 mg/kg BW)	42.65±1.35[c]	54.09±4.05[c]
MPTP + GBE (50 mg/kg BW)	56.02±2.25[d]	81.05±2.15[d]
MPTP + GBE (100 mg/kg BW)	76.01±3.72[e]	107.16±2.22[e]

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.

Table 4. Variation in the rotarod performance measured as retention time at 20 rpm in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBE (100 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (20 mg/kg BW)	51.64±0.60[b]	20.32±0.82[b]
MPTP + GBE (25 mg/kg BW)	40.03±2.90[c]	22.80±0.70[c]
MPTP + GBE (50 mg/kg BW)	50.21±2.70[d]	43.16±1.02[d]
MPTP + GBE (100 mg/kg BW)	71.07±1.35[e]	98.80±2.58[e]

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.

Table 5. Variation in the rotarod performance measured as retention time at 25 rpm in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBE (100 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	38.67±1.24[b]	12.70±0.62[b]
MPTP + GBE (25 mg/kg BW)	22.02±1.32[c]	29.16±1.03[c]
MPTP + GBE (50 mg/kg BW)	34.06±2.41[d]	41.05±1.04[d]
MPTP + GBE (100 mg/kg BW)	56.04±2.41[e]	91.65±1.32[e]

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.

100 mg/kg BW of GBE treated mice showed better retention time than 25 and 50 mg. 7 day treatment with 100 mg/kg GBT in MPTP – induced mice was significantly better than 3 day treatment. Furthermore, no differences were observed between GBE, and control group.

Tables 6-10 show the rotarod performance by MPTP-mice at various rpm (5, 10, 15, 20 and 25) on treatment with *G. biloba* gold nanoparticles on 3rd and 7th days. MPTP-induced mice on 3rd and 7th day exhibited significant decrease in the retention time on the rod when compare to control animals indicating a loss of motor coordination. Treatment with GBGNPs significantly improved the retention time as compared to MPTP- mice on both 3rd and 7th days. The 5 mg and 10 mg of GBGNPs treated mice showed similar and better retention time than 500 µg and 20 mg/kg also produce the significant retention time than the other doses. 7 day treatment was better than 3 day treatment. Furthermore, no differences were observed between GBE, and control group.

Table 6. Variation in the rotarod performance measured as retention time at 5 rpm in MPTP- mice treated with *Ginkgo biloba* gold nanoparticles (GBGNPs) on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBGNPs (20 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	73.05±1.25[b]	36.20±1.32[b]
MPTP + GBGNPs (500 µg/kg BW)	101.25±1.54[c]	104.45±2.57[c]
MPTP + GBGNPs (5 mg/kg BW)	125.94±4.65[d]	126.40±3.55[d]
MPTP + GBGNPs (10 mg/kg BW)	121.67±4.35[e]	126.95±3.34[e]
MPTP + GBGNPs (20 mg/kg BW)	123.98±5.94[f]	128.53±4.83[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.

Table 7. Variation in the rotarod performance measured as retention time at 10 rpm in MPTP- mice treated with GBGNPs on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBGNPs (20 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	62.55±1.35[b]	25.82±1.45[b]
MPTP + GBGNPs (500 µg/kg BW)	90.25±3.22[c]	105.89±1.38[c]
MPTP + GBGNPs (5 mg/kg BW)	118.65±2.62[d]	121.06±3.65[d]
MPTP + GBGNPs (10 mg/kg BW)	113.50±3.45[e]	119.90±2.01[e]
MPTP + GBGNPs (20 mg/kg BW)	115.73±5.22[f]	120.68±4.99[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.

Table 8. Variation in the rotarod performance measured as retention time at 15 rpm in MPTP- mice treated with GBGNPs on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBGNPs (20 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	58.53±1.35[b]	23.82±1.90[b]
MPTP + GBGNPs (500 µg/kg BW)	52.08±3.15[c]	86.17±1.95[c]
MPTP + GBGNPs (5 mg/kg BW)	68.28±2.65[d]	92.90±1.93[d]
MPTP + GBGNPs (10 mg/kg BW)	64.55±3.06[e]	86.98±2.93[e]
MPTP + GBGNPs (20 mg/kg BW)	66.02±2.07[f]	89.70±3.19[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.

Table 9. Variation in the rotarod performance measured as retention time at 20 rpm in MPTP- mice treated with GBGNPs on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBGNPs (20 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	51.64±0.60[b]	20.32±0.82[b]
MPTP + GBGNPs (500 µg/kg BW)	24.70±1.67[c]	53.86±1.72[c]
MPTP + GBGNPs (5 mg/kg BW)	46.22±1.65[d]	63.05±1.88[d]
MPTP + GBGNPs (10 mg/kg BW)	44.17±3.61[e]	59.41±2.94[e]
MPTP + GBGNPs (20 mg/kg BW)	42.16±2.14[f]	62.05±3.81[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.

Table 10. Variation in the rotarod performance measured as retention time at 25 rpm in MPTP- mice treated with GBGNPs on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	180.00 ± 0.00[a]	180.00 ± 0.00[a]
GBGNPs (20 mg/kg BW)	180.00 ± 0.00[a]	180.00 ± 0.00[a]
MPTP (10 mg/kg BW)	38.67±1.24[b]	12.70±0.62[b]
MPTP + GBGNPs (500 µg/kg BW)	30.92±1.53[c]	41.30±0.82[c]
MPTP + GBGNPs (5 mg/kg BW)	42.71±3.55[d]	58.07±2.02[d]
MPTP + GBGNPs (10 mg/kg BW)	39.16±2.01[e]	55.58±3.62[e]
MPTP + GBGNPs (20 mg/kg BW)	35.55±1.41[f]	53.32±5.85[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.

Hang test

Table 11 shows the results of neuromuscular strength in MPTP-mice on treatment with GBE on 3rd and 7th days. The hanging time taken by MPTP-mice was significantly reduced as compared to control. Treatment with GBE improved the hanging time as compared to MPTP- mice on both 3rd and 7th days. 100 mg/kg BW of GBE treated mice showed better retention time than 25 and 50 mg. 7 day treatment was better than 3 day treatment. Table 12 shows the results of neuromuscular strength in MPTP-mice on treatment with GBGNPs on 3rd and 7th days. The hanging time taken by MPTP-mice was significantly reduced as compared to control.

Table 11. Hang test in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	29.74 ± 0.18[a]	29.84 ± 0.73[a]
MPE (100 mg/kg BW)	29.12 ± 0.00[a]	29.36 ± 0.48[a]
MPTP (10 mg/kg BW)	9.55±0.70[b]	5.93 ± 0.32[b]
MPTP + GBE (25 mg/kg BW)	11.64±0.38[c]	15.03±10.6[c]
MPTP + GBE (50 mg/kg BW)	14.85±0.41[d]	18.35±1.12[d]
MPTP + GBE (100 mg/kg BW)	17.40±0.43[e]	19.2±0.58[e]

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.

Table 12.Hang test in MPTP- mice treated with GBGNPs on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	29.74 ± 0.18[a]	29.84 ± 0.73[a]
GBGNPs (20 mg/kg BW)	29.12 ± 0.00[a]	29.36 ± 0.48[a]
MPTP (10 mg/kg BW)	9.55±0.70[b]	3.21±0.32[b]
MPTP + GBGNPs (500 µg/kg BW)	11.41±0.78[c]	13.28±1.13[c]
MPTP + GBGNPs (5 mg/kg BW)	20.52±1.02[d]	22.86±1.32[d]
MPTP + GBGNPs (10 mg/kg BW)	18.78±1.01[e]	19.56±1.46[e]
MPTP + GBGNPs (20 mg/kg BW)	19.85±1.86[f]	20.48±1.83[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.

Administration of GBGNPs increased the hanging time as compared to MPTP-mice. The 5 mg and 10 mg of GBGNPs treated mice showed similar and better retention time than 500 µg and 20 mg/kg also produce the significant retention time than the other doses. Administration of GBGNPs treated animals showed better hanging time than GBE. 7 day treatment was better than 3 day treatment.

Narrow beam maze test

Table 13 & 14 shows the results of narrow beam test in MPTP-mice on treatment with GBE and GBGNPs 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. Treatment with GBE and GBGNPs showed significant decrease in the crossing time when compared to MPTP-mice. 100 mg of GBE treated mice showed better retention time than 25 and 50 mg. The 5 mg and 10 mg of GBGNPs treated mice showed similar and better retention time than 500 µg and 20 mg/kg also produce the significant retention time than the other doses. Administration of GBGNPs treated animals showed better hanging time than GBE. 7 day treatment was better than 3 day treatment.

Table 13. Narrowbeam Walk test in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	3.00±0.02[a]	3.02±0.01[a]
MPE (100 mg/kg BW)	3.01±0.05[a]	2.99±0.06[a]
MPTP (10 mg/kg BW)	23.20±1.15[b]	35.11±1.10[b]
MPTP + GBE (25 mg/kg BW)	15.02±1.50[c]	14.80±1.31[c]
MPTP + GBE (50 mg/kg BW)	13.64±0.62[d]	12.81±0.30[d]
MPTP + GBE (100 mg/kg BW)	11.31±1.10[e]	10.16±0.66[e]

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.

Table 14. Narrow beam Walk test in MPTP- mice treated with GBGNPS on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	3.00±0.03[a]	3.02±0.01[a]
GBGNPs (20 mg/kg BW)	3.01±0.02[a]	2.98±0.05[a]
MPTP (10 mg/kg BW)	23.20±1.15[b]	35.11±1.10[b]
MPTP + GBGNPs (500 µg/kg BW)	14.10±1.88[c]	13.18±1.47[c]
MPTP + GBGNPs (5 mg/kg BW)	9.31±0.60[d]	8.16±0.71[d]
MPTP + GBGNPs (10 mg/kg BW)	10.45±0.43[e]	10.20±0.53[e]
MPTP + GBGNPs (20 mg/kg BW)	11.32±0.50[f]	11.38±0.46[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.

Akinesia

Table 15. shows Initial movement impairment was measured by akinesia test. Administration of MPTP toxicity caused impaired ability to initiate movement (akinesia) as compared to control mice on 3rd and 7th days. Oral administration of GBE could significantly attenuate MPTP induced akinesia on both 3rd and 7th days. 100 mg of GBE treated mice showed better retention time than 25 and 50 mg. 7 day treatment was better than 3 day treatment. Moreover no significant changes were observed between control and GBE alone treated mice.

Table 15. Akinesia test in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	2.00±0.10[a]	2.00±0.10[a]
GBE (100 mg/kg BW)	2.01±0.06[a]	2.01±0.06[a]
MPTP (10 mg/kg BW)	10.12±0.15[b]	10.52±0.17[b]
MPTP + GBE (25 mg/kg BW)	4.10±0.05[c]	4.16±0.08[c]
MPTP + GBE (50 mg/kg BW)	4.12±0.08[d]	4.16±0.10[d]
MPTP + GBE (100 mg/kg BW)	5.14±0.12[e]	5.22±0.15[e]

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.

Table 16. Akinesia test in MPTP- mice treated with GBGNPS on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	2.00±0.06[a]	2.00±0.11[a]
GBGNPs (20 mg/kg BW)	2.00±0.08[a]	2.00±0.12[a]
MPTP (10 mg/kg BW)	10.12±0.15[b]	10.52±0.17[b]
MPTP + GBGNPs (500 µg/kg BW)	4.15±0.05[c]	4.21±0.09[c]
MPTP + GBGNPs (5 mg/kg BW)	5.56±0.12[d]	5.76±0.16[d]
MPTP + GBGNPs (10 mg/kg BW)	5.48±0.10[e]	5.65±0.12[e]
MPTP + GBGNPs (20 mg/kg BW)	5.51±0.11[f]	5.57±0.10[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.

Table 16. Administration of MPTP toxicity caused impaired ability to initial movement (akinesia) as compared to control mice on 3rd and 7th days. Treatment with GBGNPs significantly increased the movement speed as compared to MPTP-mice. The 5 mg and 10 mg of GBGNPs treated mice showed similar and better movement than 500 µg and 20 mg/kg also produce the significant movement than the other doses. Administration of GBGNPs treated animals showed better movement than GBE. 7 day treatment was better than 3 day treatment.

Catelepsy

Table 17 shows Impairment in movement coordination was observed by catalepsy test on 3rd and 7th days. MPTP induced toxicity mice showed significant impairment (increased time to move) in correction of an externally imposed posture (catalepsy) as compared to control mice on 3rd and 7th days. Treatment with GBE showed significant increase in the movement (lesser time) when compared to MPTP- mice. 100 mg of GBE treated mice showed better retention time than 25 and 50 mg. 7 day treatment was better than 3 day treatment. Table 18 MPTP induced toxicity mice showed significant impairment (increased time to move) in correction of an externally imposed posture (catalepsy) as compared to control mice on 3rd and 7th days.

Table 17. Catelepsy test in MPTP- mice treated with GBE on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	1.85±0.06[a]	1.85±0.06[a]
GBE (100 mg/kg BW)	1.82±0.02[a]	1.83±0.03[a]
MPTP (10 mg/kg BW)	8.01±0.11[b]	8.34±0.12[b]
MPTP + GBE (25 mg/kg BW)	4.18±0.09[c]	4.25±0.11[c]
MPTP + GBE (50 mg/kg BW)	5.16±0.10[d]	5.19±0.10[d]
MPTP + GBE (100 mg/kg BW)	5.28±0.11[e]	5.36±0.12[e]

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.

Table 18. Catelepsy test in MPTP- mice treated with GBGNPS on 3rd and 7th days

Groups	3rd Day (s)	7th Day (s)
Control	1.85±0.06[a]	1.85±0.06[a]
GBGNPs (20 mg/kg BW)	1.82±0.02[a]	1.83±0.03[a]
MPTP (10 mg/kg BW)	8.01±0.12[b]	8.34±0.16[b]
MPTP + GBGNPs (500 µg/kg BW)	4.23±0.10[c]	4.25±0.12[c]
MPTP + GBGNPs (5 mg/kg BW)	5.48±0.12[d]	5.56±0.13[d]
MPTP + GBGNPs (10 mg/kg BW)	5.34±0.10[e]	5.35±0.11[e]
MPTP + GBGNPs (20 mg/kg BW)	5.36±0.11[f]	5.38±0.14[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.

Treatment with GBGNPs showed significant increase in the movement (lesser time) when compared to MPTP- mice. The 5 mg and 10 mg of GBGNPs treated mice showed similar and better movement than 500 µg and 20 mg/kg also produce the significant movement than the other doses. Administration of GBGNPs treated animals showed better movement than GBE. 7 day treatment was better than 3 day treatment. In general, the entire behavioral test indicates that the MPTP induced toxicity in mice showed delayed response for the experiments. 200 mg of GBE treatment mice showed reverse the response when compared to MPTP. Among the GBGNPs treatment the 5 and 10 mg treatment shows the similar and better effect than the other treatment groups. 20 mg of GBGNPs also showed a better effect. Since 5, 10 and 20 mg dose of GBGNPs showed a better effect on behavior, the lower dose of 5 mg GBGNPs was fixed as the optimum dose for further work. Moreover no significant changes were observed between control and GBE, GBGNPs alone treated mice.

DISCUSSION

In the present study, the behavioural effect of the antiparkinsonian drug *G. biloba* and *G. biloba* gold nanoparticles. The results indicate a significant variation in behavioural patterns of control and experiment groups. MPTP is also a neurotoxin that produces a parkinsonian syndrome in both humans and experimental animals (Dauer and Przedbroski 2003). The MPTP mouse model of Parkinson's disease is thought to mimic more closely the behavioral pathology of Parkinson's disease, compared to the 6-OHDA rat model, and is currently the model of choice. Mice exhibit Parkinson's-like symptoms following systemic injection of the pyridine toxin MPTP, which produces a loss of striatal dopamine (DA), a nerve terminal markers and, at higher doses, death of DA neurons in the substantia nigra (Arulkumar and Sabesan, 2011). The use of a broad range of motor tasks allowed for the collection of information on different motor parameters in MPTP-induced mice such as the time required to initiate (akinesia) and execute (bradykinesia) a movement, muscle strength, gait patterns, narrow beam walking and coordinated motor performance in freely moving or exercise-driven conditions (Viaro *et al.,* 2010). The rotarod test has been extensively used to measure the motor co-ordination in experimental animals. The rotarod test, which requires animals to balance and walk on a rotating cylinder, is a widely used test to measure coordinated motor skills (Kelly *et al.,*1998), that has also been employed in the MPTP mouse model. It is stated that MPTP caused significant behavioural manifestations. In the present study, the MPTP regimen showed impaired rotarod performance (significant reduction) assessed by rotarod test with various rpm and the treatment of GBE and GBGNPs in MPTP treated mice significantly brought the retention time in rotarod. Previous study reported that, MPTP lesioning reduced rotarod performance in both male and female mice but gait was selectively impaired only in males (Antzolulatos *et al.*,2010). Zafer *et al.,* (2003) reported that the rotarod test has indicated that vehicle injection has not caused any deterioration of motor performance in the rats, while MPTP-induced animals have shown depletion in locomotion, low stereotypic events and poor-coordination. Rozas *et al.,* (1998) reported that MPTP regiment has been shown to produce severe impairment of walking in rotating rod even after 100 days of post-treatment with acute dose of MPTP. Treatment with GBE and GBGNPs significantly reverse the retention time. Simmilar findings were reported with EGB 761 treatment have reduced response time, via effects on attention, memory or motor activity (Smith *et al.,* 1996). Locomotor ability was assessed by narrow beam walking test. In the present study, MPTP-alone treated mice were observed to have a significant increase in the duration to cross the beam. Treatment with GBE and GBGNPs significantly reduced the crossing time. Previous study reported that, the motor function impairments observed on the beam walking task mice treated with acute and sub chronic dosing regimens of MPTP, were reported to display impairments in limb coordination, stride length and motor function at 1-2 week post MPTP-administration (Fernagut *et al.,* 2002; Ordonez-Librado *et al.,* 2008). Similar findings were reported that, MPTP-induced mice increase in duration to traverse the beam in Parkinson's in pit X3- deficient aphakia mice, a novel genetic model for the disease (Hwang *et al.,* 2005; Carter *et al.,* 1999) have reported that progressive motor deficits in Huntington's disease- transgenic mice model. Similar findings were observed in the present study.

Neuromuscular strength was assessed by Hang test. In the present study, the MPTP –induced mice showed significant reduction in the hang time and treatment with GBE and GBGNPs significantly improved the hang time. Previous study, reported that a significant reduction in hang time was found in MPTP injected animals. The hang test is the indicator for neuromuscular strength (Mohanasundari *et al.,* 2006). Initial movement impairment and impairment in movement coordination were measured by akinesia and catelepsy tests. In the present study, administration of MPTP caused impaired ability to initiate movement (akinesia) as compared to control mice. MPTP- regimen animals were noted to exhibit hind-limb weakness, delayed motor initiative (akinesia), postural instability and action tremor, similar findings were reported by Quinn *et al.,* (2007) in MPTP- induced mice. Behavioural assessment of akinesia in animal models of PD by means of the stepping test performance to resemble limb akinesia and gait problems seen in PD patients (Olsson *et al.,* 1995; Tseng *et al.,* 2005). Stepping test appears to be a highly useful assay for MPTP induced changes subsequent to down regulation of dopaminergic neurons in the SN (Blume *et al.,* 2009). In the present study, the treatment with GBE and GBGNPs significantly reverse the behavioural impairements induced by MPTP- regimen. Extracts from the green leaves of the G. *biloba* tree appear to be clinically effective with beneficial effects on neuroprotection, cardiovascular function and cerebral information processing. In accordance, a variety of studies have been published showing the learning- and memory-enhancing effects of standardized *G. biloba* extracts (GBEs) (containing 24% flavonoid and 6% terpenoid) in animal research (Continella 1985; Porsolt 1990; Jayaprakash *et al.,* 2010), as well as in clinical and healthy subjects (Kleijnen1992). Rocher *et al.,* (2011) reported that the neuroprotective effect of EGb 761 was associated with an improvement of spatial memory. Several research groups have shown that *G. biloba* extracts have diverse effects on improvement of mood and cognitive performance, and protection against memory deficits and central nervous system disorders (DeFeudis and Drieu, 2000; Polich and Gloria, 2001; Trick *et al.,* 2004). Administration of *G. biloba* extract improved consolidation of spatial learning, motor performance and reversed the learning deficits exhibited in aged rats (Klin *et al.,* 2009). Protective effect of *G. biloba* extracts in MPTP neurotoxicity is further revealed by improvement of locomotor activity (Yang *et al.,* 2001). The locomotor deficits observed in MPTP neurotoxicity were improved after exposure to EGb 761, probably because of a partial protection of striatal DA levels. Locomotor effects are not just a functional improvement, because it has been clearly demonstrated that EGb 761 treatment reverses the neurodegeneration of nigrostriatal dopaminergic neurons produced by MPTP neurotoxicity. (Rojas *et al.,* 2012). Ahmad *et al.,* (2005) reported that administration of *G. biloba* extract improved locomotor activity by increasing the density of tyrosine hydroxylase-positive neuronal bodies in the substantia nigra against 6-OHDA neurotoxicity.

Conclusion

It is conclude that *G. biloba* extract, attenuates the behavioural impairments like locomotor acitivty, motor co-ordination, muscular strength in a mice model of Parkinsonism, and it may be useful for the management of neuropathic pain. When compare to GBE the GBGNPs showed better improvement in all behaviour tests.

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