International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
October - November, 2016, Vol. 5, No.6, pp 2397-2404
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

NEUROPROTECTIVE ROLE OF MUCUNA PRURIENS IN PARKINSON'S DISEASE MODEL SYSTEM

Babita Singh[1], Shivani Pandey[1*], Rajesh Verma[2], Seema Singh[3], Abbas Ali Mahdi[1]

1. Department of Biochemistry, KGMU, Lucknow, India.
2. Department of Neurology, KGMU, Lucknow, India.
3. Department of Pulmonary Medicine, KGMU, Lucknow, India.

***Corresponding author's Email:** drshivani111263@gmail.com

(Received: July 30, 2016; **Accepted:** September 18, 2016**)**

ABSTRACT

Parkinson's disease (PD) is a second most common neurodegenerative disease and characterized by the progressive degeneration of the dopaminergic (DA) pathway. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes damage to the DA neurons. Treatment for this disease is still under investigation. Mucuna pruriens (Kivach), is a traditional herbal medicine, used in India since 1500 B.C., as a neuroprotective agent. In this present study, we have evaluated the therapeutic effects of M. pruriens seed extract (MPSE) in Parkinsonian mouse model. Experimental study comprised of four groups having Swiss albino mice (30-45grams) grouped as follows: Control (A), MPTP (B), MPSE (C), and MPTP+ MPSE (D), 6 mice in each group. Experimental mice were given M. pruriens seed extract treatment orally 48mg/kg bodyweight for one month with prior use of 15mg/kg b.w of MPTP treatment for two weeks. After the treatment, behavioral study was performed and assessment of Neuroprotective effect was studied via enzymatically and other molecular parameters. We observed a significant reduction in activity of tyrosine hydroxylase (TH) positive neurons in the substantia nigra (SN) region of brain, after treatment with MPTP and this activity was considerably restored by the use of MPSE. Our result suggested that MPSE treatment reduces the oxidative stress; prevent dopaminergic neurodegeneration through increase in numbers of TH positive neurons, thereby proving its antioxidant, neuroprotective and neurogenic properties. Results of this study support further investigations on this plant, as possible therapeutic intervention against Parkinson's disease.

Keywords: Parkinson's disease (PD), M. pruriens, Neurodegeneration, Neurogenesis.

INTRODUCTION

Parkinson's disease (PD) is a second most frequent neurological disorder, characterized by a selective loss of dopaminergic neurons in the substantia nigra (SN) region of ventral midbrain, causing a consequent reduction of dopamine (DA) levels in the striatum. Loss of dopamine supply to striatum causes imbalance with neurotransmitters like acetylcholine and DA, resulting in PD symptoms. Four typical characteristic symptoms observed in PD patients are tremor, rigidity, akinesia, and dyskinesia [1, 2]. The three main strategic developments in drug discovery that have advanced the progress in therapeutic management of PD patients have focused on the alleviation of motor symptoms by the use of dopaminergic mimetics, the development of novel nondopaminergic drugs for symptomatic improvement, and lastly, the discovery of neuroprotective compounds that have disease modifying effects in PD [3, 4]. The pathogenesis and etiology of PD are not completely understood, accumulating evidence suggests that glial activation-derived oxidative stress increases the risk of developing PD [5]. In vivo

and in vitro 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) models of PD have shown that key enzymes involved in the production of reactive oxygen species (ROS) are upregulated in damaged areas and thereby contribute to the death of DA neurons [6-9]. Extensive study of various models mimicking key features of PD has outlined important cellular factors of dopaminergic cell death, including neuroinflammation, oxidative stress, mitochondrial dysfunction, and excitotoxicity[10, 11]. Although no model has thus far been able to reiterate all the pathological features of PD[12], the neurotoxic models have proved themselves to be a admirable tool for developing novel therapeutic strategies and assessing the efficacy and adverse effects of symptomatic treatments of PD[13]. All current therapies are aimed at symptomatic management. Medication is often the main aspect of therapy, and its main focus lies in correcting the shortage of dopamine[14]. 'Gold standard' drug is levodopa[15]. This is most common course of action for Parkinson's disease treatment and involves the administration of a synthetic version of levodopa to increase the amount of dopamine in the brain, thus combating the effects of the disorder. Another treatment option is dopamine agonists (DAs), which have longer plasma elimination half-lives than levodopa[16]. DAs (e.g. bromocriptine, pergolide, lisuride, cabergoline, apomorphine, ropinirole and pramipexole) are able to reduce 'off' time and often allow lowering of levodopa dosage, reducing dyskinesia[14, 16]. Many drugs, including synthetic versions of natural substances, have number of side effects that can result from their chronic use. Dizziness upon standing, low blood pressure, nausea, vomiting, and uncontrollable muscle movement [17] are just a few of the identified side effects accredited to the drug.

Currently, there is no therapy clinically available that delays the neurodegenerative process itself, and therefore modification of the disease course via neuroprotective therapy is an important unmet clinical need. Thus, understanding of the pathophysiology and etiology of the disease at cellular and molecular levels and finding molecular targets for neuroprotective/disease-modifying therapy is the crucial issue in the field of basic PD research. "Neuroprotection" aims to slow disease progression and secondary injuries by halting the loss of neurons[18] or may be by promoting generation of new neurons (neurogenesis) which has not been explored in PD. Nevertheless, despite advances toward this goal, all current treatments are symptomatic; none halt or retard dopaminergic neuron degeneration. An initial good response to symptomatic pharmacological treatment declines with time, and severe side effects develop. Ayurveda, the most ancient Indian traditional medicine system ('Ayus'-Life, 'veda'- Knowledge or Science; Ayurveda means the Science of Life) provides an approach to prevent or treat different diseases by a large number of medical procedures and pharmaceuticals[19]. Mucuna pruriens, commonly known as 'cowhage plant' or 'velvet bean'or 'kapikacho' or 'kevach' in Hindi, is the most popular drug in the Ayurvedic and Unani system. The genus Mucuna, belonging to the Fabaceae family, sub family Papilionaceae, includes approximately 150 species of annual and perennial legumes. Mucuna pruriens is just one of several therapies popular in India and China for Parkinson's disease[20]. M. pruriens seeds contain significant amounts of levodopa. The endocarp of Mucuna pruriens is non-toxic and is 2-3 times more potent than synthetic leavodopa in controlling hyperprolactinemia[1] motor symptoms of Parkinson's disease animal models[21]. Mucuna pruriens has also shown to exhibit neuroprotective effect by increasing brain mitochondrial complex-I activity and significantly restoring dopamine and norepinephrine levels in Parkinsonism animal model[22]. Therefore, in view of the above mentioned multiple beneficial qualities of Mucuna, in this study, an effort has been made to explore the neurogenetic potential and neuroprotective effect of Mucuna pruriens seed extract in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's disease in mice model.

MATERIALS AND METHODS

Animals

Swiss albino male mice (8-10 weeks old, 30-45g) were used for the study. Animals were obtained from the breeding colony of IITR (Indian Institute of Toxicological Research) Lucknow and were used throughout the study with the consent of the Institutional Animal Ethics Committee in accordance with the CPCSEA guideline. All the mice were maintained on Hindustan Lever LTD (Mumbai; India) Pellets diet and water *ad libitium*. The cages were kept in temperature and humidity controlled room with 12-hr light–dark cycle.

Plant material

Prepared Ethanolic seed extract of Mucuna pruriens were purchased from Natural remedies- Banglore, for treatment of animals.

Experimental Design

Male Swiss albino mice with weight 30-45 grams were divided and put into 4 groups with six animals in each group.

Group A (Control) received normal saline water 10 ml/kg body weight orally.

Group B (MPTP) was injected i.p. with MPTP (15mg/kg b.wt.)23 for15 consecutive days.

Group C (MPSE) received 48 mg/kg body weight24 of Mucuna pruriens seed extracts (MPSE) orally for a period of one month.

Group D (MPTP+MPSE) received MPTP treatment for 15 days thereafter MPSE treatment for 30 consecutive days.

Neuro-behavioral studies were performed again to understand motor skill abnormalities by Spontaneous loco-motor activity (SLA) in each group at the end of complete treatment and animals were sacrificed by euthanasia under ketamine anaesthesia.

NEUROBEHAVIORAL ANALYSIS

Spontaneous locomotor activity (SLA)

Spontaneous locomotor activity in mice was carried out using computerized Actimot (TSE, Germany) following the method as described by Ali *et al.* [25]. Effect on different parameters including total distance travelled, resting time, stereotypic time, time moving and rearing was studied in the control and treated groups.

Grip strength

A computerized grip strength meter (TSE, Germany) was used to measure the forelimb grip strength in the control and treated animals following the standard procedure as described by Terry *et al.* [26].

BIOCHEMICAL ANALYSIS

Oxidative stress associated study

To assess, free radical mediated effects of MPTP neurotoxicity and scavenging potential of Mucuna pruriens seed extracts, estimation of lipid peroxidation (LPO), Superoxide dismutase (SOD) and Catalase was carried out in tissue homogenate [ten percent (w/v)] of striatum region of mice brain. LPO was measured by estimating malonaldialdehyde (MDA) levels following the method of Ohkawa *et al.* [27]. Catalase activity was determined spectrophotometrically by the method of Aebi28. SOD activity was determined spectrophotometrically according to the method of McCord and Fridovich [29].

IMMUNOHISTOCHEMICAL STUDIES

Immunohistochemical studies were carried out following the method of Goslin *et al.* [30]. This anaysis was carried out in the substantia nigra region of brain. Briefly, mice were anesthetized and perfused with 150 ml of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) followed by 250 ml of ice cold 4% paraformaldehyde in PBS for fixation of tissues. Brains were removed and post fixed in 10% paraformaldehyde in PBS and sampleswere kept in 10%, 20%, and 30% (w/v) sucrose in PBS. Serial coronal sections of 20-µm thickness were cut on a cryomicrotome (Microm HM 520, Labcon, Germany), incubated with primary (Tyrosine hydroxylase, Sigma, USA, 1:200) and secondary antibodies (biotinylated peroxidase linked, Sigma USA, 1:400) and processed as per protocol. The intensity of tyrosine hydroxylase (TH) positive neurons in striatal region of brain was determined using a computerized image analysis system (Leica Qwin 500 image analysis software) as described by Shingo et al31. Computerized analysis enabled to assess the percent area of a selected field that was occupied by TH positive neurons. Apoptotic neurons analysis was done by caspase-3 activity. Sections were incubated for 24 h with caspase -3 (indicator of apoptosis) primary monoclonal antibody (1:300) followed by incubation in peroxidase-linked secondary antibody for 2 h at room temperature. Sections were transferred onto gelatinized glass slides, dehydrated, cleared, mounted, cover slipped, and then visualized under fluorescence microscope.

Statistical analysis

All data were expressed as means + standard error. The test of one-way variance (ANOVA) followed by Student Newman Keuls test Compare experimental vs. Control in InStat3 package program was used to detect the significant difference between the treated groups and the control. The p-value less than 0.05 were considered statistically significant.

RESULTS

Effect of *M. pruriens* seed extract on Neurobehavioral study

To assess the motor skill abnormalities caused by neurotoxicant MPTP and to see the efficacy of MPSE, we have studied neurobehavioral changes by Spontaneous locomotor activity (SLA) and grip strength test. A significant decrease 67.09% ($p < 0.001$) was found in locomotor activity in MPTP treated mice (group B) (Fig. 1-a) as compared to control mice (group A), which was restored by MPTP+MPSE group (D) 42.33% as compared to MPTP treated group (B). However, only MPSE treated mice (group C) exhibited no significant change in the motor activity when compared to control group (A).

The grip strength was found to be significantly decreased in mice treated with MPTP (53%) compared to control group (Fig. 1-b). An improvement in grip strength (22%) was observed in mice simultaneously treated with MPTP+MPSE in comparison to those treated with MPTP only as assessed by their potential to hold the bar. No significant change on the grip strength was observed in mice treated with MPSE only when compared with control group. The results are summarized in Fig. 1

Oxidative stress associated study

MPTP treatment produced significant changes in oxidant parameter (LPO) and antioxidant parameters (SOD and Catalase) as compared to control ($p < 0.001$). MPSE administration in MPTP treated animals (group D) brought the levels of SOD, Catalase levels close to control values (Fig. 2 b, c). Activities of enzymes were attenuated in group D as compared to group B. Statistical significance between groups D and A was observed ($p < 0.001$) in MDA (Fig. 2 a) and Catalase levels (Fig. 2 c). No significant change was observed between the control (A) and MPSE (C) group. Results are given in the Fig. 2

Immunohistochemical studies

Effect on tyrosine hydroxylase (TH) Immunoreactivity

Quantification of immunoreactivity (IR) using image analysis exhibited decreased TH expression in sections treated with MPTP as compared to controls. TH is the rate-limiting enzyme and responsible for Dopamine biosynthesis. MPTP is a specific toxin and damages the dopamine producing neurons in the substantia nigra (SN) which was viewed under microscope in all experimental groups (Fig. 3). In MPTP treated mice, number of surviving TH-immunoreactive (ir) neurons were significantly less (42.5%) as compared to control group (A). MPTP + MPSE group (D) exhibited a significant increase (88%) in TH-immunoreactive neurons when compared to MPTP group (B) ($p < 0.001$). The animals treated with only MPSE (group C) have shown increase considerable TH-ir neurons as compared to control normal saline treated animals (group A). The higher number of TH-ir neurons in SN of MPTP + MPSE (D) suggests the recovery and neuroprotective action of MPSE on dopaminergic neurons. No significant change in TH immunoreactivity was observed in mice treated with MPSE only as compared to controls.

Apoptosis analysis by Caspase -3 activity

In order to assess the effect of MPSE treatment on apoptotic cell death, we carried out labeling of activated caspase 3 (indicator of apotosis). Quantitative analysis shows that significant increase in activated caspase 3 positive cells in MPTP treated group (14 + 3.18) when compared to control mice (6 + 1.41). MPTP + MPSE treated mice exhibited significant decrease (9.1 + 1.83) in caspase 3 positive cells when compared with MPTP treated mice.

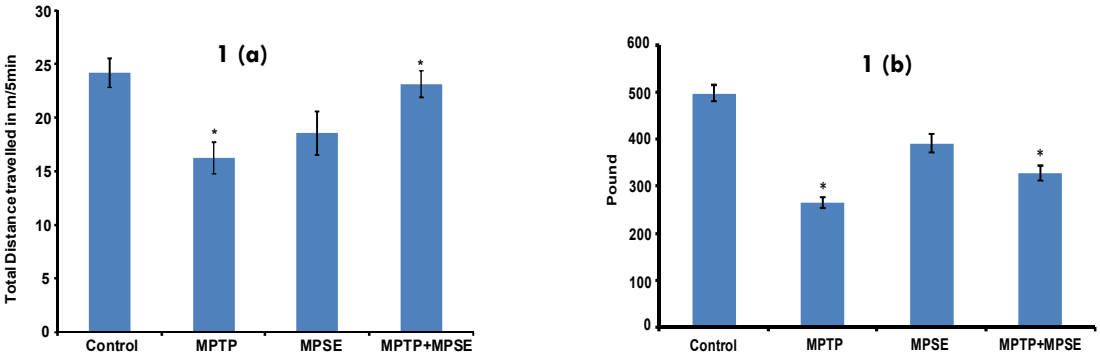

Figure: 1(a) and (b) Showing the Effect of M. pruriens seed extract on Neurobehavioral study.

* compared to control group, p- value <0.05 considered as significant.

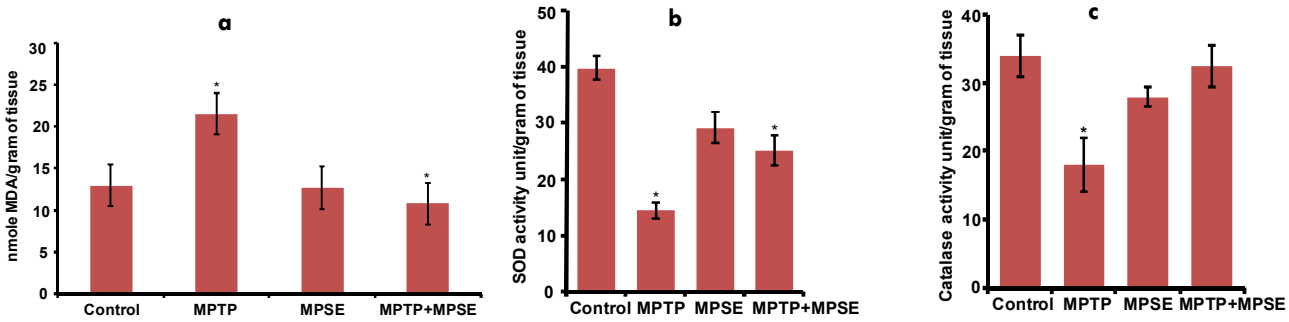

Figure: 2 (a), (b) and (c) Showing the Effect of M. pruriens seed extract on Oxidative stress parameters.

* compared to control group, p- value <0.05 considered as significant.

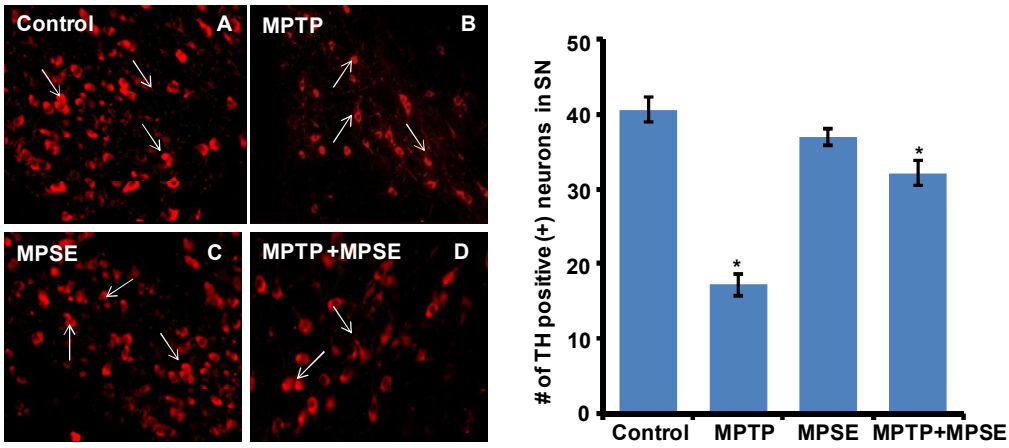

Figure: 3 TH-immunoreactive (TH+) counts in the Substantia nigra pars compacta (SNpc) of Control, MPTP, BME and MPTP+BME treated mice. Representative photomicrographs of TH-immunoreactive positive counts neurons in the SNpc of all groups. Arrows indicate TH + neurons. Quantification analysis suggested significantly decreased number of TH + neurons in the SNpc of MPTP-treated mice (B). Values are expressed mean ± SEM (n = 5 mice per group).

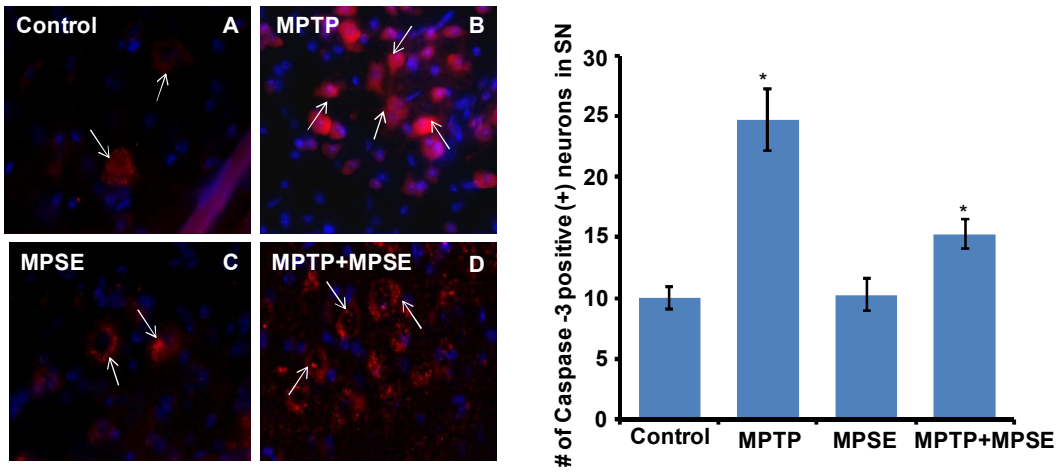

Figure: 4 MPTP exposure induces Apoptosis in the Substantia nigra pars compacta (SNpc). Representative photomicrographs showing neurons with activated Caspase 3 (Marker of cell Apoptosis) in the SNpc of all groups. Arrows indicate apoptotic neurons. Quantification analysis suggested significantly increased number Caspase-3 positive neurons in the SNpc of MPTP-treated mice and decreased number of neurons in MPTP+MPSE. Values are expressed mean ± SEM (n = 5 mice per group).

We found no significant change in only MPSE treated mice when (6.1 + 1. 94) compared with control (Fig. 4).

DISCUSSION

Neuroprotection is a mechanism based approach. Several mechanisms of neuronal injury or neuro-degeneration have been proposed including increased excitotoxicity, neuroinflammation, formation of free radicals, mitochondrial dysfunction and inhibition of protein synthesis[3]. These factors may not be sequential but certainly are interlinked. There are several management options for the early treatment of PD. Neuroprotective therapy of Parkinson disease (PD) is still theoretical, but it is based on that dopaminergic neurons in the substantia nigra can be protected from the degenerative process that causes premature cell death and depletion of dopamine, leading to the development of PD. However, no treatment for PD has been proven to be neuroprotective. Research directions include investigation into animal models of the disease and potential usefulness of gene therapy, stem cells transplant and neuroprotective agents such as nanoparticles and herbals drugs.

MP seeds are currently used in Indian ayurvedic medicine for the treatment of PD[34]. MP Seed extract is known to contain the dopamine precursor L-DOPA, which is thought to underlie the anti-PD effects[35].

Results of our study revealed that in the MPTP treated animals moving time and grip strength test were decreased, whereas retention time was increased. A reduction in locomotor activity and motor performance in MPTP treated animals has been observed, which could be strongly linked to the degree of deterioration and loss of dopaminergic cell loss. However, the animals treated with M. *Pruriens* seed extract (MPSE) have shown marked protection in the neuro-behavioral activity. L-dopa obtained from Mucuna pruriens produced better results than synthetic levodopa, in animal models[36]. Our results are in favor with other studies of M. Pruriens[37, 38] who found that M. pruriens significantly improved motor function in 6-OHDA-lesioned rats as compared to synthetic levodopa. Clinical and preclinical studies on Mucuna pruriens have substantiated claims on its efficacy and safety in PD and there are indications that it is more effective than the synthetic levodopa in reducing dyskinesias. M. pruriens seeds contain significant amounts of levodopa. The endocarp of Mucuna pruriens is non-toxic and is 2-3 times more potent than synthetic leavodopa. The possible mechanism involved in neuroprotective action of Mucuna pruriens seed extract due to presence of additional compounds (such as genistein, gallic acid, unsaturated acids, nicotine, bufotenin, harmin alkaloids, lecithin, etc) that might be improve locomotion and other motor performance.

The results of the present study clearly demonstrates that MPTP results in oxidative damage to mice brain, as evidenced by significant increase in brain malondialdehyde (MDA – an end product of lipid per-oxidation) whereas decrease in antioxidant status in brain. There is growing evidence that generation of reactive oxygen species in the SNpc neurons are implicated in the neuronal death in PD[33]. Dopaminergic neurons provide fertile environment for the generation of ROS, as the metabolism of DA produces hydrogen peroxide and superoxide radicals, and auto-oxidation of DA produces DA-quinone[39], a molecule that damages proteins by reacting with cysteine residues. MPTP oxidized to a toxic molecule, MPP+ (1-methyl-4-phenylpyridinium) by monoamine oxidase and inhibits the mitochondrial complex I in the electron transport chain and thereby disrupts the flow of electrons resulting in decreased ATP production and increased generation of ROS[40]. There are similar reports claiming oxidative damage in nervous tissue in PD disease[41, 42]. An effective antioxidant agent should be capable of augmenting intracellular concentrations of not only Super Oxide Dismutase (SOD), but also Catalase in finally reducing lipid peroxidation. Seeds of M. pruriens possess antioxidant, hypoglycemic, lipid lowering and neuroprotective activities[43] because it contains the alkaloids, mucunine, mucunadine and a number of other bioactive substances[44]. Our results also demonstrate that lipid peroxide levels were significantly high and low level of antioxidant in the MPTP treated animals, which may be due to increased oxidative stress. The improvement in anti-oxidant activity after MPSE treatment may be due to the reduction of oxidative stress. Our results are in concurrence with earlier reports that M. *pruriens* is a known adaptogen and its alcoholic extract reduces lipid peroxidation and maintains the levels of glutathione and SOD activity[45]. M. *pruriens* seeds are rich source of L-DOPA and its metabolites. Therefore, increase in dopamine level in the brain with M.

pruriens seed extract treatment may cause reduction in the oxidative stress.

Treatment with MPTP causes reduction of TH rate limiting enzyme in dopamine synthesis pathways[46]. The effect of MPSE on restoring the functional viability of dopaminergic neurons in substania nigra was studied by Tyrosine hydroxylase (TH) using monoclonal antibody against TH. The result of the present study showed that in MPTP treated mice, number of surviving TH positive neurons were significantly less and MPSE group exhibited a significant increase in TH-immunoreactive neurons. The increase in number of striatal DA neurons following MPSE treatment can also be associated with the ability of MPSE to prevent DA degradation or possibly decreased DA reuptake. The result of this study supports the previous study[46, 47]. The enhanced numbers of Dopaminergic neurons in present study, showed the protective effect of MPSE on restoring cell loss. Further, this could be due to the reduction in autoxidation of DA by enhancement of antioxidant enzymes activity.

CONCLUSIONS

The study emphasizes the importance of holistic approach of Ayurveda in using the *Mucuna pruriens* in treatment of PD. Conclusively, *M. pruriens* seed extract treatment appear to slow or reverse MPTP mediated impaired Oxidative stress, increases cell proliferation through the inhibition of apoptosis. MPSE administration also improved MPTP induced locomotor deficits. Further studies may provide an approach to understand the mechanisms involved in treating PD with lesser adverse effects.

Acknowledgement

Council of science and technology, Uttar Pradesh (UPCST), Lucknow for financial support and Indian Institute of Toxicology Research (IITR) Lucknow, for carrying out the experimental work and other support.

REFERENCES

1. Dauer W & Przedborski S. (2003). Neuron. 39: 889-909
2. Behari M, Achal K & Srivastavaa P. (2005). Parkinsonism Relat Disord. 11: 221-226
3. Liu SM, Li XZ, Huo Y & Lu F. (2012). Phytomedicine.19: 631-638
4. Schapira AHV, Bezard E, Brotchie J, Calon F, Collingridge GL, Ferger B, Hengerer B, Hirsch E, Jenner P, Spampinato U & Davidai G. (2006). Nature Reviews Drug Discov. 5: 845-854
5. Koutsilieri E, Scheller C, Tribl F, & Riederer P. (2002). Parkinsonism and Related Disorders. 8: 401-406
6. Liberatore GT, Jackson-Lewis V & Vukosavic S. (1999). Nature Medicine. 5:1403-1409
7. Choi DK, Pennathur S & Perier C. (2005). The Journal of Neuroscience. 25: 6594-6600
8. Wu D, Teismann P & Tieu K. (2003). Proceedings of the National Academy of Sciences of the United States of America. 100: 6145-6150
9. Gao H, Liu B, Zhang W & Hong J. (2003). The FASEB Journal. 17:1954-1956
10. Singh S & Dikshit M. (2007). Brain Research Reviews. 54: 233-250
11. More SV, Kumar H, Kim IS, Koppulla S, Kim BW & Choi DK. (2013). CNS & Neurological Disorders Drug Targets. 12: 680-697
12. Cannon JR & Greenamyre GT. (2010). Recent advances, Progress in Brain Research. 184: 17-33
13. Fox SH & Brotchie JM. (2010). Progress in Brain Research. 184: 133-157
14. Fahn S. (2008). In: Nass R, Przedborski S, editors, Parkinson's Disease, Elsevier; Amsterdam
15. Brooks DJ. (2008). Neuropsychiatr Dis Treat. 4: 39-47
16. Rascol O, Lozano A, Stern M & Poewe W. (2011). Mov Disord. 26: 1072-1082
17. Ramya KB & Thaakur S. (2007). Anc Sci Life. 27: 50-55
18. Seidl SE & Potashkin JA. (2011). Frontiers in Neurology. doi: 10.3389/fneur.2011.00068
19. Pandey GS & Chunekar KC. (1996). Bhavprakash nighantu Chaukhamba Vidhyabavan, Varanasi. 357-359
20. Ovallath S & Deepa P. (2013). Mov Disord. 28: 566-568
21. Manyam BV, Dhanasekaran M & Hare T. (2004). Phytother Res.18: 706-612
22. Tripathi Yamini B & Upadhyay AK. (2002). Phytother Res. 16: 534-538
23. Gibrat C, Saint-Pierre M, Bousquet M, Levesque D, Rouillard C & Cicchetti F. (2009). Journal of Neurochem, 109: 1469-1482
24. Kasture S, Pontis S, Pinna A, Schintu N, Spina L, Longoni R, Simola N, Ballero M & Morelli M. (2009). Neurotoxin Res. 15: 111-122
25. Ali MM, Mathur N & Chandra SV. (1990). Indian J Exp Biol. 28: 653-656
26. Terry AV, Stone Jr JD, Buccafusco JJ, Sickles DW, Sood A & Prendergast MA. (2003). J. Pharmacol. Exp. Ther, 305: 375-384
27. Ohkawa H, Ohishi N, & Yagi K. (1997). Annals of Biochemistry, 95: 351-358
28. Aebi H, Catalase in vitro. In: Packer, L. (Ed.), Methods in Enzymology 105, Academic Press, New York, 1984, 121
29. McCord JM, & Fridovich I. (1969). Journal Biol Chem. 224: 6049-6055

30. Goslin K, Schreyer DJ, Skene JHP & Banker G. (1990). Jour Neurosci. 10: 588-602
31. Shingo T, Date I, Yoshida H & Ohmoto T. (2002). J Neurosci Res. 69: 946-954
32. Tiwari SK, Agarwal S, Seth B, Yadav A, Nair S, Bhatnagar P & Chaturvedi RK. (2014). ACS Nano. 8: 76-103
33. Dias V, Junn E & Mouradian MM. (2013). Journal of Parkinson's Disease. 3: 461-491
34. Manyam BV, Dhanasekaran M & Hare TA. (2004). Phytother Res. 9: 706-712
35. Sathiyanarayanan L & Arulmozhi S. (2007). Pharmacogn Rev.1: 157-162
36. Lieu CA, Venkiteswaran K, Gilmour TP, Rao AN, Petticoffer AC, Gilbert EV, Deogaonkar M, Manyam BV & Subramanian T. (2012). Evid. Based Complement Alternat Med, 2012: 840247
37. Yadav SK, Prakash J, Chouhan S & Singh SP. (2013). Neurochem Int. 62: 1039-1047
38. Huot P, Johnston TH, Koprich JB, Fox SH & Brotchie JM. (2013). Pharmacol Rev. 65: 171-122
39. Graham DG. (1978). Molecular Pharmacology.14: 633-647
40. Nicklas WJ, Vyas I, & Heikkila RE. (1998). Life Sciences. 36: 2503-2508
41. Iczkiewicz J, Jackson MJ, Smith LA, Rose S & Jenner P. (2006). Brain Research.1118: 239-250
42. Chaturvedi RK, Shukla S, Seth K, Chauhan S, Sinha C & Shukla Y. (2006). Neurobiology of Disease. 22: 421-434
43. Sharma ML, Chandhoke N, Ray Ghatak BJ, Jamwal KS, Gupta OP & Singh GB. (1978). Indian J Exp Biol 16: 228-235
44. Misra L & Wagner H. (2007). Indian J Biochem Biophys. 44: 56-60
45. Shukla KK, Mahdi AA, Ahmad KM, Jaiswar SP, Shankwar SN & Tiwari SC. (2010). eCAM. 7:137-144
46. Aznavour N, Cendres-Bozzi C, Lemoine L, Buda C, Sastre JP & Mincheva Z. (2012). CNS Neuroscience & Therapeutics. 18: 934-941
47. Agrawal AK, Chaturvedi RK, Shukla S, Seth K, Chauhan S, & Ahmad A. (2004). Journal Chem Neuroanat. 28: 253-264.

