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Mucuna pruriens seed extract reduces oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in paraquat-induced Parkinsonian mouse model

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease which causes rigidity, resting tremor and postural instability. Treatment for this disease is still under investigation. *Mucuna pruriens* (L.), is a traditional herbal medicine, used in India since 1500 B.C., as a neuroprotective agent. In this present study, we evaluated the therapeutic effects of aqueous extract of *M. pruriens* (Mp) seed in Parkinsonian mouse model developed by chronic exposure to paraquat (PQ). Results of our study revealed that the nigrostriatal portion of Parkinsonian mouse brain showed significantly increased levels of nitrite, malondialdehyde (MDA) and reduced levels of catalase compared to the control. In the Parkinsonian mice hanging time was decreased, whereas narrow beam walk time and foot printing errors were increased.

Treatment with aqueous seed extract of Mp significantly increased the catalase activity and decreased the MDA and nitrite level, compared to untreated Parkinsonian mouse brain. Mp treatment also improved the behavioral abnormalities. It increased hanging time, whereas it decreased narrow beam walk time and foot printing error compared to untreated Parkinsonian mouse brain.

Furthermore, we observed a significant reduction in tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra (SN) and striatum region of the brain, after treatment with PQ which was considerably restored by the use of Mp seed extract. Our result suggested that Mp seed extract treatment significantly reduced the PQ induced neurotoxicity as evident by decrease in oxidative damage, physiological abnormalities and immunohistochemical changes in the Parkinsonian mouse.

1. Introduction

Mp (Fabaceae), commonly known as cowhage plant or kapikachhu, is the most popular drug in Ayurveda, the Indian system of medicine (Chopra et al., 1956). It is a climbing legume found in southern China and eastern India (Huisden, 2005). All its parts possess valuable medicinal properties. Several reports have suggested that it possesses analgesic, anti-inflammatory, anti-neoplastic, anti-epileptic and anti-microbial activities (Adepoju and Odubena, 2009; Sathiyanarayanan and Arulmozhi, 2007). Traditionally, in India, the seeds of Mp are used as a tonic and aphrodisiac for male virility. Mp has even been reported to be a rejuvenator drug having neuroprotective property (Manyam et al., 2004).

Parkinson's disease (PD) is a chronic neurological disorder caused by a progressive degeneration of nigrostriatal dopaminergic (DAergic) neurons and characterized by a slowness of movement, muscular stiffness, rigidity, tremor, poor posture, poor balance and sensory-motor integration deficits (Obeso et al., 2000). Several epidemiological studies reveal the relationship between PD and environmental factors such as rural residence (Morano et al., 1994), farming (Liou et al., 1997), drinking water from wells (Marder et al., 1998) and exposure to agricultural chemicals, pesticides, and herbicides (Semchuk et al., 1992). Among these environmental neurotoxins, several pesticides like maneb, rotenone, cypermethrin, are used to create animal models to study the mechanism of PD and its therapeutic interventions (Hirsch et al., 1991; Singh et al., 2008, 2009; Tiwari et al., 2010; Tanner, 1989). In spite of all these studies there are few reports available regarding good animal models of human PD (Tiwari et al., 2010; Prakash et al., 2013). Recently PQ induced PD model have been developed and it is considered as one of the best model to date because of the slow progression of the disease (Patel et al., 2008).

Abbreviations: DAergic, dopaminergic; IHC, immunohistochemical; ip, intraperitoneal; LPO, lipid peroxidation; MDA, malondialdehyde; Mp, *Mucuna pruriens*; NO, nitric oxide; PD, Parkinson's disease; PQ, paraquat; ROS, reactive oxygen species; SN, substantia nigra; TH, tyrosine hydroxylase.

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Recent studies suggest that free radicals also have a key role in PD (Lopez-Real et al., 2005). PQ intoxicated mice had high level of free radicals, mitochondrial dysfunctions, microglial activation, increased lipid peroxidation (LPO) and elevated nitric oxide level. PQ enhances alpha (α)-synuclein-induced disruption of membrane integrity and increases the conductance, as a result of increased oxidative stress. PQ slows down mitochondrial complex I activity and augments the microglial activation and free radical production through NADPH oxidase, which may lead to oxidative stress, DNA damage, defective energy metabolism, and cellular apoptosis (Singhal et al., 2012; Thiruchelvam et al., 2000; Uversky, 2004;).

The released hydroxyl radicals readily react with polyunsaturated fatty acids to yield lipid hydroperoxides leading to oxidative damage, DNA damage, membrane permeability and integrity loss (Gupta et al., 2010). Nitric oxide (NO) plays an important role in neurotransmitter release, neurotransmitter re-uptake and regulation of gene expression; however its excessive production leads to neurotoxicity (Dawson and Dawson, 1998). It is well known that ageing, nervous disorders, decrease in performance and some other sexual disorders are mediated by free radicals (Aitken and Clarkson, 1987).

Mp contains many micronutrients including amino acids, Zn, Se, carbohydrates (Prakash et al., 2001; Ghosal et al., 1971; Mehta and Majumdar, 1994; Panikkar et al., 1987) and various plant alkaloids (Rakshit and Majumdar, 1956; Ghosal et al., 1971). In addition the herb contains L-DOPA, an amino acid which is converted into dopamine in the brain (Misra and Wagner, 2004, 2007).

A clinical trial of commercially available Mp (HP-200) was found effective in PD (HP-200, 1995). However, the exact mechanism is still unexplored. Mp is a significant source of natural antioxidant as stated by earlier studies (Kumar et al., 2010; Tripathi and Upadhyay, 2001), thus, it is possible that this plant may act through a mechanism of free radical removal in the management of the above disorder.

In the present study we have tried to examine the possible effect of Mp seed extract on DAergic neurons against neurodegenaration. For this we studied three parameters; first changes in motor functions by narrow beam, foot printing and hanging tests, second oxidative stress occurring in nigrostriatal region and third expression of TH in the SN and striatum by immunohistochemistry performed on Parkinsonian mice.

2. Materials & methods

2.1. Medicinal plants and preparation of extracts

Mp seeds were collected from the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The extraction was carried out by the method of Uhegbu et al. (2005) using distilled water as the solvent. 20 g of powdered sample of the Mp seed was soaked in 200 ml of autoclaved distilled water, stirred for about 6 min for proper mixing and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) and the extracts were evaporated to dryness under reduced pressure and temperature (below 40 °C) in rotary vacuum evaporator (Fig. 1). The plant extract was expressed in terms of dry weight.

2.2. Acute toxicity testing

Swiss albino male mice weighing 25 ± 5 gram were randomly distributed to nine different groups having 6 animals in each group. The animals were fasted overnight and Mp was administered orally at dose level of 50, 100, 200, 400, 800, 1600, 3200, 6400 and 12,800 mg/kg body weight (wt) for 3 consecutive days. We measured the body weight and hair loss pattern of the experimental mice. The animals were also closely observed for any toxic symptoms and mortality rate according to the method described earlier (Ecobichon, 1997).

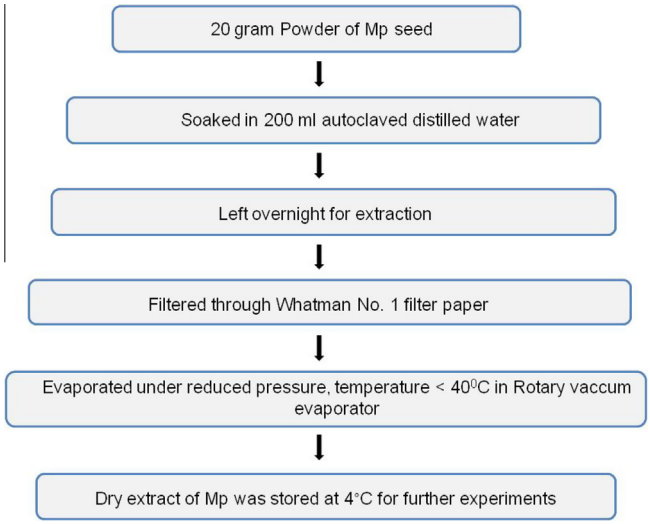

Fig. 1. Diagrammatic illustration of aqueous extract preparation of Mp seed powder.

2.3. Dose response experiment

For optimization of treatment doses of *Mucuna pruriens*, for neuroprotection in PD, initially mice were treated with 25, 50, 100, 150 and 200 mg/kg body weight. Behavioral tests were then conducted on all the groups for three weeks. Initially, we had set up an experiment for identifying the appropriate dose.

2.4. Animal treatment

Male swiss albino mice weighing 25 ± 5 gram were used in the experiment, which were obtained from the animal house of the Institute of Medical Science, BHU, Varanasi, India. Guidelines of the Institutional Ethics Committee for use of laboratory animal were followed in this study. Animals were maintained under standard conditions of temperature (22 ± 5 °C), humidity (45–55%) and light (12/12-h light/dark cycle). The animals were fed with standard pellet diet and water *ad libitum*.

Animals were randomly divided into three experimental groups ($n = 6$) as follows;

Group I: Mice were given intraperitoneal (ip) injections of saline (0.9%), this served as control
Group II: Mice were administered ip injections of PQ (10 mg/kg body wt.), twice in a week for 3, 6 and 9 weeks (Gupta et al., 2010)
Group III: Mice were orally treated with (100 mg/kg body wt.) aqueous seed extract of Mp daily (Rajasankar et al., 2007) and also received PQ (10 mg/kg body wt.) twice in a week for 3, 6 and 9 weeks.

PQ was obtained from Sigma Aldrich (St. Louis, Mo, USA). All the above treatments were performed in three sets for 3, 6 and 9 weeks to check the dependency of disease development and its treatment on time duration. At the end of each experiment (3, 6 and 9 weeks), behavioral studies were performed to understand motor abnormalities.