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Ursolic acid attenuates oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in MPTP-induced Parkinsonian mouse model

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ABSTRACT

Parkinson's disease (PD) is characterized by a slow and progressive degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc) region of brain. Oxidative stress and inflammation plays important role in the neurodegeneration and development of PD. Ursolic Acid (UA: 3β-hydroxy-urs-12-en-28-oic acid) is a natural pentacyclic triterpenoid found in various medicinal plants. Its anti-inflammatory and antioxidant activity is a well-established fact. In this paper, the neuroprotective efficiency of UA in MPTP induced PD mouse model has been explored. For this purpose, we divided 30 mice into 5 different groups; first was control, second was MPTP-treated, third, fourth and fifth were different doses of UA viz., 5 mg/kg, 25 mg/kg, and 50 mg/kg body weight (wt) respectively, along with MPTP. After 21 days of treatment, different behavioral parameters and biochemical assays were conducted. Tyrosine hydroxylase (TH) immunostaining of SN dopaminergic neurons as well as HPLC quantification of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) were also performed. Our results proved that, UA improves behavioral deficits, restored altered dopamine level and protect dopaminergic neurons in the MPTP intoxicated mouse. Among three different doses, 25 mg/kg body wt was the most effective dose for the PD. This work reveals the potential of UA as a promising drug candidate for PD treatment.

1. Introduction

PD (PD), the second most common age associated progressive neurodegenerative disorder is characterized by the loss of dopaminergic (DA) neurons, aggregation of α-synnuclein proteins and neuroinflammation (Lee et al., 2009). It is also characterized by slowness of movement, muscular stiffness, rigidity, tremor, poor posture, imbalance and sensory-motor integration deficits (Patil et al., 2014). The neuronal connection between substantia nigra (SN) and the striatum of the brain is essential for normal motor functions. If deterioration of these neurons occurs, it results in depleted dopamine Influx which leads to major pathological hallmarks of PD (Wooten, 1997). Oxidative stress and inflammatory processes in the brain and nervous system play important role in the progression of this disease (Halliwell and Gutteridge, 1985). Nigrostriatal dopaminergic neurons of the mid brain region are more susceptible towards oxidative stress induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mishra et al., 2000; Sadrzadeh and Saffari, 2004). The deleterious effect of ROS and RNS can be attenuated by detoxification of free radicals using antioxidants.

UA, a natural pentacyclic triterpenoid, is a component of certain traditional medicinal herbs and ornamental species and is also found in fruits, such as apples, prunes, cranberries, and blueberries (Liu, 1995; Neto et al., 2008). So, the cornerstone of our work was to study anti-PD potential of UA as the protective effects of UA were determined by its strong antioxidative properties (Ali et al., 2007). It has been reported to possess many biological activities including antitumor, antioxidant and anti-inflammatory properties (Wu et al., 2013). The neuroprotective ability of *Mucuna pruriens* (Mp) seed extract has been reported in our previous work (Yadav et al., 2013). We assessed and quantified each of the phytochemical of Mp by HPTLC finger printing patterns. Both L-DOPA and ursolic acid was found in significant proportions in the Mp seed extract. Overall, the Mp seed extract was found to contain 18.84% L-DOPA and 4.5% ursolic acid (data not published). This presence of ursolic

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acid in significant quantity leads us to investigate the anti-Parkinsonian effect of ursolic acid in MPTP induced mouse model. There are also reports showing neuroprotective effect of UA through the inhibition of oxidative stress and inflammation in different neurotoxic models (Lu et al., 2007; Wang et al., 2011). In this study, we have used MPTP intoxicated mouse model as MPP^+, the active metabolite of MPTP, stimulates the production of free radicals in neuronal cells and thus can be used in neurological studies to induce oxidative and/or inflammatory injury (Chinta et al., 2006; An et al., 2006; Sasaki et al., 2007).

In the present investigation, the effect of different concentrations of UA on neurobehavioral parameters and enzymatic antioxidant systems were examined. Expression of TH in the SN by immunohistochemistry and level of dopamine with reference to its metabolites were quantified.

2. Material and method

2.1. Animal treatment

Male Swiss albino mice weighing 25 ± 5 g were used in the experiment. The mice 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 libitium. Animals were randomly divided into five 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 two dose ip injections of MPTP (30 mg/kg body wt), at 16 h interval; In Groups III, IV and V: Mice were orally treated with UA (5, 25 and 50 mg/kg body wt respectively) and were also given two doses of MPTP (30 mg/kg body wt), at 16 h interval. At the end of treatment different analysis were performed to understand neuroprotective potential of UA.

2.1.1. Chemicals

Acetic acid, disodium hydrogen phosphate, glutathione (GSH), potassium chloride and sodium dihydrogen phosphate were procured from Sisco Research Laboratories (SRL; Mumbai, India). Streptavidin-peroxidase, normal goat serum and the DAB (3,3diaminobenzidine) system were procured from Bangalore Genei Pvt. India Ltd., Bangalore, India. 1-Methyl-4-phenyl-1, 2, 3, 6-tetra hydropyridine (MPTP). Folin Ciocalteau reagent, hydrogen peroxide (H_2O_2) and potassium dichromate were purchased from Merck (Darmstadt, Germany). Primary antibodies for TH were procured from Santa Cruz, Biotechnology (Santa Cruz, CA, USA).

2.2. Behavioral parameters

2.2.1. Rotarod test

In the Rotarod experiment, test animals were trained for 3 consecutive days before starting the experiment at a fixed speed (15 rpm). The time taken by mice to fall down was recorded, up to a maximum of 5 min. For each animal, the experiment was repeated four times and the average time was calculated (Manna et al., 2006). The experiment was repeated after the completion of the treatment and time taken by the mice to fall was recorded.

2.2.2. Hanging test

Mice were placed on a horizontal grid and supported until they hold the grid. The grid was inverted so that the mice hang upside down. Animals were allowed to stay on the grid until they fall, and hanging time was recorded as described earlier (Mohanasundari et al., 2006).

2.2.3. Narrow beam walking test

Narrow beam walking test was performed to access the motor coordination requiring balance and stability. Animals were trained to walk on a stationary wooden narrow flat beam (L100 cm W1 cm) which was placed at a height of 100 cm from the floor. Time taken to walk the beam from one end to the other was recorded as described earlier (Pisa, 1998).

2.3. Sample collection

After the completion of behavioral tests, mice were euthanized by cervical dislocation followed by decapitation to ensure minimum pain. The brains were removed on ice to maintain enzyme activity and were frozen immediately following removal. Further, brains were dissected on ice cold conditions to isolate nigrostriatal tissue and were stored at −80 °C until the biochemical estimations were performed (Kumar et al., 2010a).

2.4. Biochemical parameters

2.4.1. Lipid peroxidation

Lipid peroxidation in the nigrostriatal tissue of mice brain was estimated according to a method described earlier (Ohkawa et al., 1979) with slight modifications. In brief, 10% homogenate was mixed with 10% SDS solution followed by the addition of 20% acetic acid. Finally 0.8% TBA was added and the reaction mixture was incubated in a boiling water bath for 1 h. The assay mixture was cooled, centrifuged and absorbance of the supernatant was read at 532 nm against control. LPO levels were expressed as nmole of MDA/mg protein.

2.4.2. Nitrite estimation

Nitrite level was estimated by using standard procedure (Granger et al., 1996). In brief, 10% tissue homogenate was incubated with ammonium chloride and mixed with Griess reagent. The reaction mixture was incubated at 37 °C for 30 min, and the absorbance of the supernatant was recorded at 540 nm. The nitrite content was calculated using a standard curve for sodium nitrite (10–100 μM) in terms of μmole/ml.

2.4.3. Catalase activity

Catalase activity was assayed by measuring the rate of decomposition of hydrogen peroxide spectrophotometrically (Kumar et al., 2010a). Briefly, tissue homogenate was incubated with potassium dichromate and acetic acid (1:3) for 10 min in boiling water bath and OD was taken at 570 nm. The enzymatic activity was measured in μmole/min/mg protein.

2.5. Measurement of dopamine, DOPAC and HVA

The levels of DA and its metabolites (DOPAC and HVA) were estimated from the isolated nigrostriatal homogenate using a HPLC—electro chemical detection (ECD) system (Kim et al., 1987; Krishnamurthy et al., 2011). In brief, the brain tissue samples were homogenized in 0.17 M perchloric acid using a Polytron homogenizer. Homogenates were centrifuged at 33,000 × g (BiofugeStratos, Heaureas, Germany) at 4 °C. The supernatant 20 μl was injected into a HPLC pump (Model 1525, Binary Gradient Pump) having a C18 a column (Spherisorb, RP C18, 5 mm particle size, 4.6 mm i.d. 250 mm at 30 °C) connected to an ECD (Model 2465, Waters, Milford, MA, USA) at a potential of +0.8 V with a glassy carbon working electrode vs. a Ag/AgCl reference electrode. The mobile phase consisted of 32 mM citric acid, 12.5 mM disodium hydrogen orthophosphate, 1.4 mM sodium octylsulfonate, 0.05 mM EDTA and 16% (v/v) methanol (pH 4.2) at a flow rate of 1.2 ml/min. The chromatogram