ABSTRACT

Studies on Neurochemical Alterations and Oxidative Stress Mediated Dopaminergic Degeneration in the MPTP Model of Parkinson’s Disease: Protective Effect of Withanolides and Curcuminoids

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Parkinson's disease (PD) is a progressive, multifactorial neurodegenerative disorder, characterized by motor disabilities and associated behavioral impairment. The pathological hallmark of the disease is the loss of nigrostriatal dopamine (DA), driven by mitochondrial dysfunction, oxidative stress and inflammation. Despite advancement in understanding of the pathophysiology of PD through pre-clinical and clinical studies; treatment of PD is still enigmatic. Currently available conventional drugs only provide symptomatic relief without affecting retardation of the disease progression and concomitantly exert severe side effects in the long-term use. Pertinent to it, therapeutic drug candidates derived from medicinal plants have gained considerable attention worldwide due to their pleiotropic mode of action and safe in long term use for various disorders including PD. With this pre-context, the present research work comprehensively explored and provided new insight regarding the mode of action of withanolides and curcuminoids isolated from two revered medicinal plants of Indian System of Medicine (ISM), particularly used in Ayurveda namely *Withania somnifera* (L.) and *Curcuma longa* (L.) in the MPTP-induced mice model.
In the present study, active fractions from standard root extract of *Withania somnifera* and *Curcuma longa* rhizomes viz., withanolides (steroidal lactones/alkaloids) and curcuminoids (polyphenols) respectively, were extracted and quantified the marker compounds by high performance liquid chromatography (HPLC). In-vitro assays were performed to assess the total antioxidant, nitric oxide (NO) inhibition, chelation properties and monoamine oxidase-B (MAO-B) inhibition activity of the tested drug candidates. At the commencement of the study, a small dose dependent pilot study was conducted in-order to obtain the optimum dose of 1-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20x4 mg/kg; i.p.) for model development, as well as, for effective dose of withanolides (100mg/kg/day; p.o.) and curcuminoids (150mg/kg/day; p.o.) by screening their efficacy over PD biomarkers. In an exploratory study, male C57BL/6 mice were pre-treated with withanolides (100mg/kg/day; p.o.) and curcuminoids (150mg/kg/day; p.o.) for one week, followed by four intra-peritoneal (i.p.) injections of MPTP (20x4mg/kg) at 2 h intervals with subsequent administration of both drugs for consecutive two weeks. Concurrently, deprenyl was also administered as standard MAO-B inhibitor/ anti-parkinsonian drug (3 mg/kg/day; p.o.) for two weeks.

Furthermore, in-order to investigate the neuroprotective effect of withanolides and curcuminoids, present series of study has comprehensively evaluated their effect over PD biomarkers; dopamine (DA) and its metabolites, MAO-B, tyrosine hydroxylase (TH) protein expression; nor-adrenaline (NE) and 5-hydroxy tryptamine (5-HT) content; behavioral paradigms, oxidative stress markers, mitochondrial impairment and inflammatory responses in the MPTP-induced mice brain. The results of the present study suggested that oral administration of withanolides and curcuminoids significantly
alleviated the MPTP-induced cellular toxicity by ameliorating the DA and TH content may be in-part through modulation of catecholamine biosynthesis and IL-1β and TNF-α cytokine levels; attenuating GFAP, iNOS and nNOS expression; restoring compromised endogenous antioxidant like GSH and enzymes, SOD and catalase concomitant inhibition in lipid peroxidation and protein carbonyl generation. In-addition, withanolides and curcuminoids profoundly exerted protective effect over mitochondrial complexes and thus prevent ATP depletion in the MPTP+drug treated groups. Collectively the effect of withanolides and curcuminoids on MPTP-induced dopaminergic degeneration was also reflected in behavioral studies where marked improvement was observed in employed behavioral tests including rota-rod, open field, hang time and tail suspension tests, which validates that withanolides and curcuminoids possess neuroprotective activity.

Taken together, the results of the present research work demonstrated the multi-targeted action of withanolides and curcuminoids in MPTP-induced mice brain, which was validated through substantial in-vitro and in-vivo experiments. The current findings provided adequate evidences that both withanolides and curcuminoids possess antioxidant, anti-inflammatory effect and protective mitochondrial activity which may in-turn prevents dopaminergic neurodegeneration against MPTP-induced toxicity. Collectively, the present findings suggests that withanolides and curcuminoids holds immense potential as a putative drug candidates for the prevention and treatment of PD due to its pleiotropic mechanism of action and warrants further research to extrapolate pre-clinical findings into clinical studies for better treatment and management of PD.