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Role of Limonene in Experimental Model of Parkinson's Disease

by

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Abstract

Parkinson's disease (PD) is one of the prevalent neurodegenerative diseases in elderly population. The symptoms of PD often begin with motor and cognitive impairment that are attributed to the dopaminergic neurons degeneration. Rotenone (ROT) is a naturally derived pesticide that is used in agriculture to control pests. It is a neurotoxin well-established for use in resembling PD symptoms in research. Limonene (LMN) is a plant derived monoterpene extracted from citrus peel with a wide range of therapeutic effects. Due to the shortage in available therapeutic agents that can cure or halt the progressive degeneration of PD, the main aim of this thesis is to scrutinize and to reach a consensus regarding the potential neuroprotective effects of LMN in a rat model of ROT-induced PD against underlying mechanisms including oxidative stress, neuro-inflammation and apoptosis. An animal model of PD was established on Wistar rats by intraperitoneal injection of (2.5mg/kg) ROT for 28 days. Modulatory functions of LMN (50 mg/kg) were investigated by performing immunofluorescence for Ionized calcium-binding adaptor protein-1 (Iba-I), Glial fibrillary acidic protein (GFAP) and Tyrosine Hydroxylase (TH) in both striatum and substantia nigra. Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) were also evaluated. Neuro-inflammation biomarkers; interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were assessed by ELISA. Immunoblotting was performed for measuring other biomarkers including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), BAX , Bcl-2, cleaved caspase-3 and caspase-9, Cytochrome C, Brain Derived Neurotrophic Factor (BDNF), mitochondrial complex-I (MC-I), α-Synuclein and NF-κB. Rotarod test was also performed to assess motor coordination. Results showed a significant loss in dopaminergic neurons following ROT administration. However, LMN treatment rescued the dopaminergic neurons from the progressive loss in both substantia nigra and striatum. LMN attenuated the activation of astrocytes and microglia observed in ROT-injected rats and decreased the raised levels of inflammatory mediators, such as COX-2 and iNOS, IL-1β, IL-6 and TNF-α in addition to suppressing NF-κB signaling pathway. LMN treatment restored the activity of antioxidant enzymes, prevented glutathione depletion and inhibited lipid peroxidation. The phosphorylated JNK and P38 kinases were downregulated in LMN pretreated rats, while p-mTOR was upregulated. Apoptosis was remarkably alleviated by LMN administration evident by the increase and reduction in anti-apoptotic and pro-apoptotic proteins respectively. Motor functions were also improved significantly in LMN treated rats. Findings reported a regressive α-Synuclein expression, improved BDNE level and a restoration of complex-1 activity following LMN administration. Given these findings, LMN mitigated ROT-induced dopaminergic neurodegeneration, which is suggestive of the protective properties of LMN in PD treatment through various biological mechanisms.

Keywords: Parkinson's disease, Rotenone, Limonene, Neurodegeneration, Dopaminergic neurons, Phytochemical, Oxidative stress, Neuro-inflammation