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Therapeutic Potential of *Withania somnifera* Extract for Parkinson's Disease: Impact on Neuronal Synaptic Integrity and Hormonal Regulation

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Abstract

Parkinson's Disease (PD), a multifactorial movement disorder, is neuropathologically characterized by age-dependent neurodegeneration of the dopaminergic neurons in *Substantia nigra*. In PD patients, the hypothalamic dysfunction results in disruption of pituitary hormone secretion. Several genetic mutations contribute to the pathogenesis and advancement of PD. Among them, synaptic protein mutations play a critical role. The treatment of PD, using L-Dopa and other classes of drugs such as dopamine agonists, monoamine oxidase inhibitors, and anticholinergic agents, provides only symptomatic relief. Long-term use of these drugs produces side effects and adds to oxidative stress by producing more free radicals, which contribute to disease progression. Synaptic reconstruction and neurite regeneration are the critical steps for the retrieval of normal brain function. So, the therapeutic approach for discovering new effective neuroprotective agents that would enable neurite regeneration and establishing functional synapses is vital. Recently, emphasis has been given to the herbal medicines and their bioactive ingredients to develop alternative therapies to PD, which could provide efficient neuroprotective support to existing drugs. *Withania somnifera* root extract, containing steroidal alkaloids and steroidal lactones, has shown excellent potential in PD treatment. Even though *Withania somnifera* offers nigrostriatal dopaminergic neuroprotection by modulating oxidative stress and apoptotic machinery, the exact mechanism of neuroprotection is yet to be elucidated. Withanolide A, one of the active compounds in *Withania somnifera,* facilitated the neurite outgrowth and reconstruction of synapses in PD models. Additionally, this plant extract appears to alleviate endocrine-associated modifications in PD patients. This review summarizes the major findings on the use of *Withania somnifera* and its biochemical influences in neuroprotection, regulating endocrine function and maintenance of synaptic integrity of neurons.

Keywords: Hypothalamic Dysfunction, Luteinizing Hormone, Neuroprotection, Parkinson's Disease, Synaptic Protein, Synaptic Reconstruction, *Withania somnifera*

1. Introduction

Parkinson's Disease (PD), the second most common progressive neurodegenerative disease, is a chronic, progressive neurological movement disorder characterized by involuntary tremulous action, rigidity and co-ordination difficulties. PD involves the selective degeneration of dopaminergic neurons of *Substantia nigra*, an area influencing all involuntary movements[1]. Pathologically, PD is identified by the presence of aggregates known as Lewy bodies comprised of α-synuclein, parkin, ubiquitin and neurofilaments[2]. Understanding the fundamental mechanisms underlying the development and progression of PD pathology

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is significant for the development of neuroprotective therapies. The exact cause for PD is unknown; still, it could be due to a combination of genetic and environmental factors. Approximately 95% of PD is not linked with genetics, and is referred as sporadic PD. But in some forms of PD mutations in genes encoding α-synuclein (PARK 1), parkin (PARK 2), UCHL-1 (PARK 5), PINK 1 (PARK 6), DJ-1 (PARK 7), LRRK2 (PARK 8) and ATP13A2 (PARK 9) have been reported, and these are referred to as familial PD[3].

The neuronal communication, mediated through the regulated calcium-dependent exocytosis of synaptic vesicles at the presynaptic active zones of nerve terminals, is essential for synapse modulation[4]. A variety of synaptic proteins regulates synaptic functions. Alteration in synaptic proteins in various model organisms has provided evidence for their critical roles in synaptic function including regulation of neurotransmission[5]. Synaptic proteins also play an essential role in maintaining the structure and organization of synapse, exocytosis, and endocytosis. The variations in the expression pattern of these synaptic proteins can modify neuronal functions[6]. Synaptic transmission necessitates the proper targeting of proteins to the synaptic vesicles and the assembly of synaptic terminals[7]. This review focuses on the synaptic dysfunction in Parkinson's disease, and treatment options based on the extract of *Withania somnifera* to maintain synaptic integrity and alleviate associated anterior pituitary endocrine dysfunction.

2. Relation between PD and Synaptic Dysfunction

The association between the synaptic protein homeostasis and the development of PD has been elucidated[8]. There is evidence to the effect that most of the PD-associated gene mutations are related with proteins that control the synaptic function[9]. Among the PD cases, 5-10% are accounted by the monogenic familial mutations of the genes regulating synaptic function and protein turnover. Knockdown or over expression studies of these "PD genes" have been shown to alter synaptic dysfunction, and defects in protein turnover[8]. The experimental Parkinsonism with substantial dopaminergic neuron loss has shown cellular and synaptic modifications in the striatum, which further contribute to the clinical characteristics of PD[10]. There are evidences for synaptic dysfunctions in PD ahead of the dopaminergic neuronal loss, including DA synaptic terminal degenerations[8]. Studies on post-mortem samples of PD patients show that synaptic dysfunction happens much before neuronal death[11].

The dopaminergic neurons in the *Substantia nigra* region of the brain, one of the most affected in PD, are highly branched and form excessive synaptic connections, compared to the dopaminergic neurons in the Ventral Tegmental Area (VTA- a region less affected by PD) which forms lesser number of synapses. Higher synaptic density and vulnerability to neurodegeneration indicate additional parameters for neuronal degeneration[8]. Deciphering the molecular mechanisms at the synapses could provide a critical understanding of their role in neurodegeneration[12].

3. Presynaptic Players Involved in PD

The presynaptic terminal has functional and structural elements to allow morphological and physiological alterations during the disease onset and contribute to further progression of the disease. These alterations include the synaptic protein and synaptic vesicle depletion, and neurotransmission defects. The critical genes involved in presynaptic dysfunction include α-synuclein and LRRK2, two autosomal dominant genes, and Parkin, PINK-1 and DJ-1, three autosomal recessive genes. The α-synuclein, a 140 amino acid protein on the synaptic vesicle, is linked with synaptic vesicle trafficking, SNARE complex formation, and maintaining the dopamine level[11,13]. The neurons lacking α-synuclein result in reduction of undocked synaptic vesicles, while the docked synaptic vesicle level remain unaltered[14,15]. Knockout of α-synuclein results in increased level of induced dopamine release indicating that the α-synuclein functions as a negative regulator of dopamine neurotransmission[16].

A series of studies have revealed the critical role of α-synuclein in synaptic neurotransmission, which is essential for maintaining the neurotransmitter dopamine level in the synapses. The α-synuclein over-expression leads to a decrease in the synaptic vesicle recycling pool, ultimately affecting the neurotransmission[17]. The impairment of α-synuclein leads to dopamine accumulation, which converts into highly reactive toxic moieties inducing synaptic degeneration[18]. The abnormal aggregates of α-synuclein results in Lewy body

formation and aggrevate the PD symptoms. Typically, the α-synuclein remains in an equilibrium of monomers and α-helically folded tetramers which have less tendency for the formation of aggregates[19], while under PD conditions, the ratio of tetramer and monomer declines and the unfolded monomers tend to aggregate leading to the formation of β-sheet structures of oligomers, and insoluble fibrils resulting in Lewy bodies[20].

Another significant role player in PD is LRRK2 (Leucine-rich repeat Kinase 2), a multidomain protein having kinase, GTPase, and protein-protein interaction domains with structural and functional regulatory roles at synapses[21]. Genomic studies in PD patients have revealed a direct link between LRRK2 mutation and onset of the disease; in transgenic animals, LRRK 2 mutation has shown a deficiency in striatal dopamine release and dopamine uptake[22]. LRRK2 regulates the synaptic vesicle endocytosis and SNARE complex disassembly by phosphorylating endophilin and NSF (N-ethylmaleimide sensitive factor). The association of LRRK2 with various cytoskeletal elements and presynaptic proteins bring strong evidence that synaptic dysfunction could occur due to variations in LRRK2 protein[21,23,24]. The functional inactivity of Parkin, an E3 ubiquitin ligase, is associated with the onset of juvenile PD[25]. The loss of function of the Parkin gene resulted in impaired neurotransmission by the deprivation of synaptic proteins, reduced dopamine release, and weakened synaptic plasticity[26].

PINK-1 (PTEN-induced putative kinase 1), a mitochondrial regulator that phosphorylates Parkin and Ubiquitin, is a strong candidate for early onset familial PD[27]. PINK-1 mutation leads to mitochondrial abnormalities, diminished dopamine release, and defective neurotransmission[28]. Yet another significant candidate, DJ-1, a redox-sensitive molecular chaperon, contributes to autosomal recessive early-onset PD[29]. The loss of DJ-1 results in mitochondrial dysfunction, reduced mitochondrial complex I activity, and disrupted mitochondrial homeostasis[30]. The association between α-synuclein, LRRK2, Parkin, PINK1, and DJ-1 regulates the dopamine release pathways and plays a crucial role in the onset of PD pathology[10]. Molecules that can stabilize the functioning of these proteins and regulate the dopamine level are thus the ideal choice for PD therapy.

4. PD Treatment

Currently, there is no cure for PD. Levodopa (L-Dopa), the direct biosynthetic precursor of dopamine, is the standard treatment to alleviate the clinical symptoms. L-Dopa is generally administered along with a peripheral decarboxylase inhibitor, carbidopa, to prevent peripheral conversion to dopamine, thus stabilizing the availability of dopamine and reducing the peripheral side effects[31]. Though L-Dopa generates remarkable improvement in elevating PD-associated symptoms, long-term treatment using L-Dopa is less efficient and more likely to produce side effects[32]. These changes occur because L-DOPA dosing results in the oxidative load by forming free radicals during its metabolism and progression of the disease[31]. Various other drugs such as dopamine agonists, Mono-Amine Oxidase (MAO) inhibitors, catechol-O-methyltransferase inhibitors, and anticholinergic agents also could be used in the symptomatic treatment of PD[33]. Still, none of these drugs can prevent progression of the disease. Besides, these drugs show significant side effects on continuous use. Therefore, it is essential to bring up new therapeutic agents that will reduce the neurodegeneration of PD[34].

Protecting the neuronal connectomes in areas prone to degeneration, and thus allowing the normal brain function, would be the appropriate first step towards the PD treatment. Exploring the bioactive compounds of plant origin that would protect the synapse function is one of the strategies explored[35]. The antioxidants are the key therapeutic agents used to repair the damage caused by free radicals in PD[35]. Therefore, the antioxidant-rich natural products have been used as adjuvant therapy and conventional treatment to reduce the dosage of dopaminergic drugs to reduce the adverse effects due to prolonged use of dopaminergic agents[36]. The search for novel drug candidates for PD with fewer side effects has been ended with natural products, such as medicinal herbs, plant extracts, secondary metabolites, phytochemicals, and active ingredients from lower organisms[37]. These natural products are reported to have great potential as therapeutics with neuroprotective activity, particularly in PD models.

Ayurveda, the ancient system of Indian medicine, has identified a variety of plants having antioxidant activities that could be therapeutically used for curing neurodegenerative diseases. For example, extracts prepared from *Bacopa monniera* had proved to improve human cognitive function and have an antioxidative function[38]. An extract from *Ginkgo biloba* is reported to reduce free radical levels[39]. Several more plants offer scope to be examined for their potential neuroprotective properties[40]. Among them, *Withania somnifoera* offers the most potential application in PD treatment.

5. *Withania somnifera* (Ws) Alleviates PD

Withania somnifera (L.) Dunal, popularly known as 'Ashwagandha' in Sanskrit and 'Indian Winter Cherry' in English, is a medicinal herb extensively used in Ayurveda. This plant belongs to the Solanaceae family. Even though root is most used part of the plant in Ayurvedic preparations, the leaves and stem also have shown pharmacological activities. *Ws* has stress-relieving properties equivalent to depression and anxiety drugs[41]. It has powerful antioxidant properties to scavenge and destroy the free radicals produced during aging and in many disease states[42]. This plant extract is prescribed in Ayurveda for treating neurodegenerative diseases such as AD and PD. The neuroprotective effects of the root extract of *Ws* in various PD animal models and the mechanisms of action have been studied[43-45].

The neuroprotective nature of *Ws* is mainly contributed by its capacity to enhance the dopamine and tyrosine hydroxylase levels and its ability to intensify the antioxidant potential[44]. Studies conducted on the MPTP-treated mice, a Parkinson's disease model, showed that *Ws* root extract could be used as a promising drug in reducing catecholamine levels, oxidative damage, and physiological aberrations observed in the PD[45]. The root extracts of *Withania somnifera* have shown to protect SH-SY5Y cells from MPP^+-associated toxicity (Sukumaran *et al*., unpublished data).

The administration of methanol extract of *Ws* rescued the progression and abrogated the symptoms associated with PD in LRRK2 mutants of Drosophila[46]. *Ws* influences many neurotransmitter receptors in CNS and has fewer adverse side effects. Even though *Ws* is a traditional medicine offering several health benefits, the mechanisms of action are not well understood. The beneficial effects of *Ws* could be attributed to the presence of two groups of compounds namely, steroidal alkaloids and steroidal lactones[47]. The steroidal lactones comprise a group of components called with anolides. In *Ws,* 12 alkaloids and 35 with anolides are reported, among which Withaferin A, Withanolide A, and Withanolide D are known for their pharmacological effects[48,49]. The *Ws* extract, with so many active alkaloids, steroidal lactones, and saponins, has significant antioxidant properties to rescue the neurons[43]. The chemical constituents (Figure 1) and pharmacological properties of *Ws* extracts have been extensively reviewed[47]. The foremost challenge in treating the neurodegenerative diseases with the possible therapeutics includes the incapacity to cross the Blood-Brain-Barrier (BBB). Withaferin A, with a molecular weight lesser than 500 Da, the lipophilic structure and its suitable blood/brain protein coefficient, has the potential to penetrate the BBB[50]. Studies on animal models and clinical trials have ascertained its use through both oral and intraperitoneal administrations[51,52]. Thus Withaferin A is postulated as a potential drug candidate in treating PD.

6. Synaptic Reconstruction by *Ws*

In PD treatment, the utmost essential requirement is the functional recovery of the affected brain areas. Therefore, the compounds that could protect the neuronal connectome and the synapses are the best choice for the treatment. Various studies have demonstrated the efficiency of *Ws* extracts in neurite growth. For example, the methanol extract of the plant has been shown to enhance the dendritic extension in neurons[53] and in human neuroblastoma SH-SY5Y cells[54]. Withanolide A,

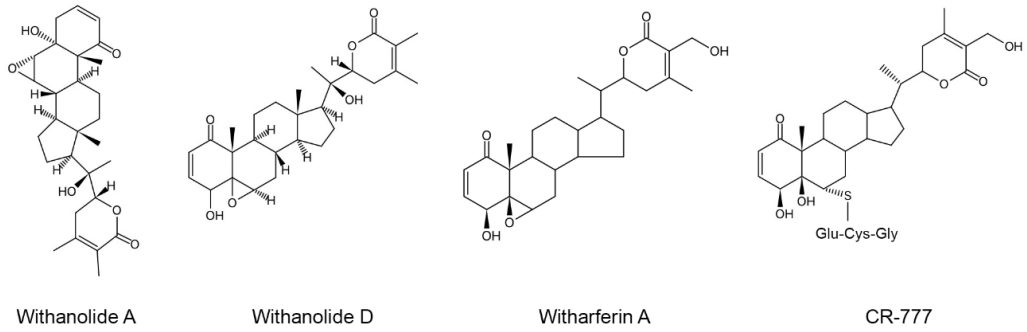

Figure 1. Chemical structure of Withanolide A, Withanolide D, Withaferin A and CR-777.

a key component of *Ws*, has been shown to enhance the outgrowth of axons of the cortical neurons[55]. Studies have shown that the Withanolide A acts to regenerate the dendrites and axons and reconstruct the pre-and post-synaptic organization of the degenerated neurons[56].

The level of Semaphorin 3A (SEMA 3A), an axonal chemorepellent, was found to be elevated during neuronal damage[57]. The treatment with *Ws* extract could reduce SEMA 3A, thus overcoming its effect on preventing neuronal regeneration by enhancing synapse formation[58]. The fungus *Beauveria bassiana* bio-convers *Ws* extract into the compound CR-777, a cysteine and glutathione derivative of Withaferin A (Figure 1), protecting the cells from α-synuclein aggregation, one of the pathological hallmarks of PD[59]. The action of *Ws* extract on neuroblastoma cells encouraged the association of autophagy and proteasomal pathways to function together to get rid of the α-synuclein clusters[60]. Recent studies have revealed that the i-extract of *Ws* has neuroprotective and neurotrophic properties by restoring the dendritic growth and rebuilding neuronal networks[61]. The postulated neuroprotective role of *Ws* is summarized in Figure 2.

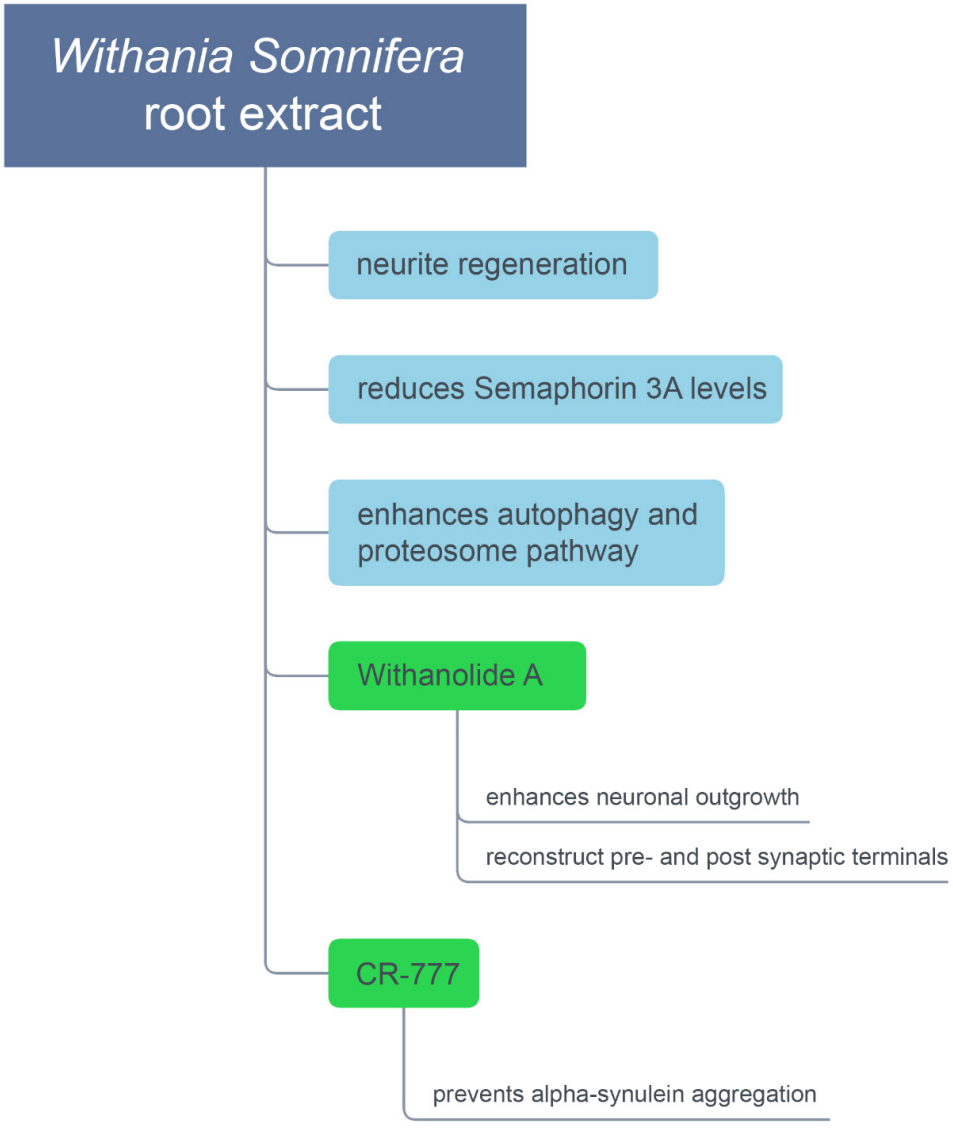

Figure 2. The neuroprotective roles of *Withania somnifera* extract (highlighted in blue) and its metabolites (highlighted in green).

7. PD and Hormonal Integration

Many symptoms and pathophysiology of PD affect the basal physiological mechanisms associated with hormonal integration. The pathological and biochemical studies have revealed that the PD patients show symptoms of hypothalamic dysfunction, followed by disturbances at the peripheral enteroendocrine cells producing neuropeptides[62,63]. In untreated PD patients unaltered levels of prolactin, Thyroid-Stimulating Hormone (TSH), Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Growth Hormone (GH) and Insulin-like Growth Factor (IGF-1) were observed while the dopaminergic treatment resulted in the deviations in the pituitary and somatotrophic system especially hypoprolactinemia and elevated GH secretion[64]. PD patients have shown testicular dysfunction, which subsequently led to a lower level of Hypothalamic-Pituitary-Gonadal (HPG) axis hormones especially LH, FSH and testosterone[65-67]. The decreased level of testosterone could result in depression, erectile dysfunction and low sperm count. In PD patients the overwhelming rise of dopaminergic metabolizing enzymes could have impact on the secretion of HPG axis hormones[68]. In addition, the anterior pituitary endocrine dysfunction is also prevalent in PD patients[69].

The postmenopausal women with PD have been reported to have an altered hypothalamic dopaminergic system which regulates LH secretion[70,71]. The dopamine receptor stimulator, bromocriptine, has been shown to have undesirable effect in regulating pituitary hormone secretion in PD patients[72]. Some of the neuroendocrine anomalies that are associated with PD consist of disturbance in melatonin secretion, instabilities of glucose metabolism and insulin resistance. The PD patients have shown glucose intolerance up to a level of 80%, revealing a strong association of PD with type 2 diabetes mellitus (T_2DM)[73]. The PD phenotypes like postural instability, gait difficulties, cognitive impairment and disease progression are more related with T_2DM as the dopaminergic therapies did not improve the axial motor symptoms and cognitive impairment[74]. Deciphering the complexity of neuroendocrine interactions during the disease progress could have vital implications on diagnosis and therapeutic approaches[62].

8. Effect of *Ws* on Hormonal Regulation

A majority of the endocrine-associated alterations in PD, discussed above, could be regulated by *Ws*. Age-related decrease of testosterone can enhance the susceptibility to PD. Strong experimental evidence has established the role of *Ws* on the endocrine system by increasing the level of serum testosterone and LH[75,76]. *Ws* extracts improved the levels of testosterone and LH in studies conducted with opioid-addicted adult male rats[77], and enhanced the balance between LH and FSH in infertile men[75,78]. Studies using the water extract of *Ws* on immortalized rat hypothalamic GnV-3 cell line revealed that it would upregulate the Gonadotropin-Releasing Hormone (GnRH)[79]. The anxiolytic property of *Ws* is attributed to its controlling effect on the HPA axis and thus increasing the testosterone level[80]. Experiments on diabetic male rats revealed the regulatory effect of *Ws* extract on the levels of gonadal hormones, particularly progesterone[81,82]. *Ws* has been shown to have a protective effect on T_2DM by normalizing hyperglycemia through improving insulin sensitivity in fructose-fed rats[83] and in Non-Insulin-Dependent Diabetes Mellitus (NIDDM) rats[84]. These data suggest that the overall pharmacological benefit of *Ws* in PD patients could be two-sided, both at neuronal and endocrine.

9. Future Directions

In spite of the prevalent studies on the use of *Ws* extracts in neurodegenerative diseases, there is a need for more in-depth research to explicate the definite mechanism of its action. Further phytochemical studies have to be performed to purify the secondary metabolites that are responsible for the potential therapeutic properties and develop commercial formulations. The pharmacological activities, therapeutic effect and mode of action of the metabolites in the various parts of *Ws* plant need additional evaluation for the clinical use. The potential therapeutic target and the pathways that could be affected by the bioactive metabolites in *Ws* need to be elucidated. Even though the neuroprotective effect of the bioactive compounds in treating PD without side effects is known for long, the ability of such compounds to cross the blood-brain-barrier needs to be extensively studied. More systematic studies on these plant extracts and their active ingredients aiming at regulation of nerve cells and the endocrine system will provide a much-needed novel drug discovery platform for PD.

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11. Conflict of Interest

The authors declare that they have no conflict of interest.

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