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The Role of Mucuna Pruriens and Withania Somnifera in the Neuroprotection and Treatment of Parkinson's disease.

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Abstract

The fundamental purpose in treating patients with Parkinson's disease (PD) is dual (i) to increase the dopamine content in striatum (ii) to prevent further ventral midbrain degeneration of the surviving dopaminergic neurons in the substantia nigra pars compacta (SNpc) region. Currently most of the PD drugs like levodopa medication, monoamine oxidase B inhibitor (MAO-B), dopamine agonist and catechol-O-methyl transferase (COMT) inhibitor contribute to the former and provide only symptomatic relief. Although compounds like Levodopa (L-DOPA) recover dopamine content in the striatum but their long-term usage causes progressive decrease in drug response, motor fluctuations, dyskinesias and ultimately causes drug-induced toxicity. Additionally, these drugs fail to prevent the progressive degeneration of dopaminergic neurons in the SNpc of midbrain region in PD. Thus this existing drug has shifted the focus onto additional alternative therapeutic approaches that involve Ayurvedic natural products that could provide independent therapy or offer neuroprotective support to the dopaminergic neurons in the SNpc region. The current review describes the neuroprotective and therapeutic utility of such Ayurvedic natural products including herbal extracts like *Mucuna pruriens* (Mp) and *Withania somnifera* (Ws) in isolation or in combination, with potential application in PD.

Introduction

Parkinson's disease

PD is a progressive neurological disorder of the central nervous system with growing layers of complexity. It is characterised by the classical motor symptom associated with accumulation of Lewy bodies (LB) and loss of dopaminergic neurons in the SNpc of the mid brain region. On the other hand, the symptomatology of PD is heterogeneous, with clinically significant non-motor characteristic. Likewise, the pathology of PD involves extensive regions of the central nervous system, a variety of neurotransmitters, and protein aggregates other than LB. The etiology of PD remains unknown, but probable risk of developing PD is not only due to environmental factors. Instead, PD seems to result from a complicated interplay between environmental factors and genetic factor that adversely affects the plentiful primary cellular processes. The major complexity of PD is arises due to an inability to make a definitive diagnosis marker at the earliest stages of the disease and difficulties in the management of symptoms at later stages. In addition, nowadays there are no treatments available that slow the progression of neurodegenerative process [1].

Levodopa and other dopaminergic medications significantly improve the motor symptoms and quality of life of patients with PD in its early stages. But, usually after a few years of dopaminergic therapy, when the effective period has passed, patients become gradually more weakened despite of the availability of intricate combination of antiparkinsonian treatments. Eventually, they suffer from motor symptoms (speech impairment, abnormal posture, gait and balance problems), nonmotor signs (autonomic dysfunction, mood and cognitive impairment, sleep problems, pain) and/or drug-related side effects (especially psychosis, motor fluctuations, and dyskinesias) due to Dopa-resistance. Certainly, one of the most prominent examples of the success of modern neuropharmacology has been served by the antiparkinsonian treatment. However, alone or joint with other interventions, none of the presently available antiparkinsonian therapies, offers an entirely satisfying strategy for managing patients with PD on a long-term basis: many motor and nonmotor features are resistant to currently available treatments, several treatments induce disabling adverse reactions, and quality of life and life expectancy remain abnormal. Furthermore, several of the most recent treatment innovations are too expensive or too complex for many patients. There remains a need for efficacious, safe, and cheap symptomatic, neuroprotective, or restorative antiparkinsonian treatment to deal with these unmet medical needs in the management of PD. Therefore, the current antiparkinsonian therapy cannot be considered as ideal with regard to both efficacy and safety [2].

Ayurvedic medications have a lot of opportunity for patients with PD. Although the pharmacological actions of specific herbs like Atmagupta (Mucuna Pruriens) are being found helpful, complete treatment requires proper lifestyle and daily routine changes that appease the patient's imbalance and in the long

term creates the harmony with the patient's constitution. In this approaches following the yogic model, patients should be encouraged to look within to discover the fundamental psychological and religious components that contributing to their condition. The cornerstone of Ayurvedic healing understands where a person is out of harmony on the physical, emotional and spiritual levels since disease is the end result of living out of harmony with one's constitution. With the help of this understanding, a person can take the necessary actions that bring back his or her harmony and healing. Ayurvedic viewpoint teaches us that the pleasant-sounding or harmonious person with a purely sattvic nature, does not experience disease or disorder. Similarly, the sick person, who cultivates a sattvic mind, brings back quick healing to their body constitution. Therefore, all PD patients should be encouraged to reduce their different types of stress and develop practices such as meditation, which bring about peaceful mind. In addition to herbs future Western scientific examination of Ayurvedic curative will have to go further than the pharmacological actions of various herbs, and discover the effects of Ayurvedic lifestyles, and Yogic practices applied as part of a treatment regimen. Whereas Mp has an expected allopathic effect which is easy to determine, result can be planned to look at complete treatment programs and not simply individual components. The effectiveness of Ayurvedic treatment depends upon the internal and subtle energies of our being, goes beyond the pharmacological regimen. Although these qualities are not easy to isolate and examine, they can be evaluated as a whole, and it is here that genuine Ayurvedic research begins [3]. Medicinal plants like Mp and Withania somnifera (Ws) have been used in traditional Ayurvedic medicine to manage Parkinson's disease [4,5,6].

The two major herbal plants and their extract which have significant impact in recent days in the area of PD are as follows:

Mucuna pruriens (Mp)

Mucuna pruriens, also known as 'atmagupta', 'cowhage' or 'velvet bean' is found in India, Africa, the Caribbean and parts of the tropics including central and South America is a tropical legume of the family Fabaceae. Since ancient time it is well known to possess medicinal properties and is commonly used as an anti-depressant. Ayurvedic texts explain the treatment of PD with extracts of atmagupta [7]. For the treatment of PD Mp seed preparations are in contemporary use in India [8]. From the seeds of Mp L-Dopa was isolated in 1937 and with the understanding of the usefulness of L-DOPA for PD therapy, scientific interest to review on those plant which are L-DOPA rich [9]. From the phytochemical screening of the water and ethanolic extracts of Mp established the presence of various medicinal component like saponins, tannins, anthraquinones, terpenoids, flavoniods and cardiac glycosides, which are indicative of its medicinal properties [10]. Mp seeds are also rich nutritional component like carbohydrates, proteins, lipids, minerals and fiber, sterols, lecithin; sterols are also present in Mp. In Mp's seed extract L-DOPA, the dopamine precursor, accounts for nearly 7-10%. Previously, a recovery method of L-DOPA from Mp seeds by repeated hot water extraction, ion exchange separation followed by elution with 10% acetic acid was reported [11]. In vivo and in vitro studies suggest that Mp extracts are non-toxic on a daily basis oral dose of up to 600 mg/kg body weight [12]. Subramanian's study suggested that the Mp seed powder produced anti-PD effects and did not cause dyskinesia, while some adverse effects like gastric intolerance were observed perhaps due to the 'bulky' nature of the orally administered powder [13]. HP- 200 a commercially available edible formulation by Zandu Pharmaceuticals, India, consists of Mp cotyledon powder is a flavouring agent and a sweetener and is marketed by the name 'Zandopa'. HP-200 alleviates the pathophysiology of PD in 6- hydroxydopamine (6-OHDA) lesioned rats more effectively than the commercially available L-DOPA probably due to its non-L-DOPA components which could either enhance the effects of L-DOPA or have L-DOPA independent anti-PD effects. In a multicenter clinical trial it is proved that HP-200 has been proved to be better than the synthetic L-DOPA/syndopa involving 60 patients over a 12 week period [14]. Clinical study demonstrate that that Mp is a potential alternative to the conventional drug administered a concoction of powdered Mp, Hyoscyamus reticulatus seeds, Withania somnifera and Sida cordifolia roots in cow's milk, in 18 PD patients [15]. Thus these studies strongly suggest that a pharmacological agent obtained from natural sources could be harmless in its natural scene and there activity could be modulated by other important bioactive components. To optimize the therapeutic applications of Mp there are continued efforts to obtain better Mp extract. Thus in experimental models of PD most patents deal either with the extraction and fractionation of Mp seeds and/or its application either alone or in combination with other natural products. A recent patent US6106839 describes the use of an Ayurvedic composition consisting of Mp seeds (55-99% w/w), Zingiber officinalis roots (5-15% w/w) and Piper longum fruits (10-35% w/w) which alleviates motor dysfunction associated with PD [16]. This patent clearly describes that, when an effective dose of 2-6 g/day of this Ayurvedic mixture was administered on a female PD patient who was clinically diagnosed over an 11 year period (age 51-62 y), it proved to be more advantageous compared to conventional PD therapy. US7470441 is an another patent describes the serial extraction of Mp seeds with organic solvents ultimately obtaining a fraction that contains L-DOPA at a lower concentration than the pharmaceutically effective amount and at least one other active pharmacological agent [17]. If required this extract is suitable for oral, topical or parenteral application and can be applied as such, or in combination with isolated L-DOPA. Use of the Mp formulation does not present the adverse effects unlike conventional L-DOPA therapy wherein, with increasing dose, short and long term introduced side-effects such as dyskinesia claimed by the author. The clinical study presented suggests that in fluctuating patients with short duration L-DOPA response the L-DOPA in the seed formulation is sufficient to consistently induce a sustained on-period effect. This formulation of Mp contains very low amount of L-DOPA since the L-DOPA of Mp is entrenched in an organic material and also contains definite additives like ascorbic acid and citric acid, also there is improved intestinal absorption and enhanced cellular uptake. In addition, the Mp fraction showed significant

enhancement in dopamine uptake and neuroprotection against 1-methyl-4-phenylpyridinium (MPP+) and buthionine sulfoximine (BSO) toxicity in primary dopaminergic neuron cultures. A double blinded and challenging study indicated better L-DOPA tolerance and effective advantages over the existing commercial synthetic drugs in PD patients administered with the Mp formulation. Another associated patent US3253023 present a efficient method for L-DOPA isolation from a velvet bean, Stizolobium deeringianum (a subgenus of the Mucuna genus), by extracting with a dilute 1-10% organic acid solution at 10-80ºC for ~10-20 hours followed by separation, filtration and ultimately concentrating of L-DOPA [18].

Recent updates on *Mucuna pruriens*

Yadav et al shows that Mp seed extract reduces oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in paraquat (PQ) induced Parkinsonian mouse model [4]. This study demonstrates the neuroprotective effect of Mp seed extract in PQ induced parkinsonian mouse. The result from this study suggests that the Mp seeds aqueous extract has a strong antioxidant property, which helps to reduce the oxidative stress generated in PQ induced PD mice. Mp seed extract improves motor behavior, due to reduction in oxidative stress and also improves the expression of Tyrosine hydroxylase (TH) in SN and striatum of the brain in PQ intoxicated mice. Therefore, this study strongly suggests that Mp seed extract, a precious herbal plant which can be used for developing drug for treatment of PD.

Yadav et al., in 2014 also shows that Mp seed ethanolic extract has a potent neuroprotective role against the MPTP-induced PD model. The Mp seed ethanolic extract suggest a strong antioxidant potential against MPTP induced PD mouse model. Additionally, Mp treatment enhanced motor behaviour of PD mice. Mp induced the level of catecholamines and stimulates the antioxidant potential in the SNpc region. Mp treatment improved the expression of TH in the SN and striatal regions and recovered normal expression levels of iNOS and GFAP in MPTP induced PD animals. This study Suggest the Mp's tendency to support in the revival from neuronal injury and oxidative stress. Overall, this study suggest that this herbal product from India's natural medical system (Ayurveda) could be used for the development of therapeutics against PD and other neurodegenerative diseases by both reversing the symptoms and correcting the primary cause [19].

Anti-PD property of Mp is also explored in genetic model of PD in the paper entitled "Mp rescues motor, olfactory, mitochondrial and synaptic impairment in PINK1^{B9} *Drosophila melanogaster* genetic model" [20].

Mucuna pruriens shows neuroprotective activity by inhibiting apoptotic pathways of dopaminergic neurons in the PQ mouse model. Mp exhibit anti-apoptotic activity through the improvement of Bcl2 expression and turn down the level of Bax [21]. In this study Yadav et al., perform the HPTLC of Mp' s seed extract and found L-DOPA along with ursolic acid are the major component. Thus L-Dopa and ursolic acid is mainly responsible for the underlying anti-parkinsonian activity of Mp. Ursolic acid attenuates oxidative stress in SNpc region and improve the neurobehavioral activity in MPTP induced parkinsonian mouse [22].

Yadav et al., explore the anti-inflammatory activity of Mp, Mucuna pruriens reduces inducible nitric oxide synthase expression in Parkinsonian mice model [23]. This study demonstrates that the Mp has a strong anti-inflammatory property, which helps to reduce the neuroinflammation generated in PD mice model. Nigrostriatal portion of the PQ treated brain mice exhibit elevated levels of MDA and nitrite. Expression of iNOS is induced by excessive secretion of inflammatory molecules during cellular injury and infection which is the main contributor to progression of PD pathogenesis. Mp treatment improves motor behavior impairment, due to the reduction in neuroinflammation and oxidative stress in SN and also improves the expression of TH in SN region of the brain and protected the dopaminergic neurons in the PQ-intoxicated mice. The results from this study are in accordance with the previous investigations showing the role of nitric oxide in neurodegeneration and iNOS inhibitors in neuroprotection in PD. Thus Mp extract appears to be potential drug candidate in the Parkinson's neuroprotection.

Various studies have shown that in the central nervous system, Mp can alter the immune components like Tumor Necrosis Factor- α (TNF-α), Interleukin-6 (IL-6), Interferon-λ (IFN-λ), Interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS) and Interleukin-2 (IL-2). In PD, Mp can also alter the activity of the nuclear transcription factor NF-kB which plays a vital role by regulating the expression of various proinflammatory cytokines. Consequently, by altering these above mentioned cytokines along with nuclear transcription factors NF-kB, Mp can protect or prevent the progression degeneration of dopaminergic neurons in PD [24].

Recently Rai et al have published an article entitled "*Mucuna pruriens* Protects against MPTP Intoxicated Neuroinflammation in Parkinson's Disease through NF-kB/pAKT Signaling Pathways". In this published paper, aqueous extract of Mp has significantly protected the dopaminergic neurons from MPTP toxicity by its anti-inflammatory activity This study has suggested that along with TH and DAT immunoreactivity, aqueous extract of Mp significantly ameliorate the neuroinflammatory processes as well as restore the behavioral and biochemical abnormalities. They have also suggested that NF-kB and Akt pathway might be responsible for the fundamental mechanism behind Mp action. Thus, the potent anti-inflammatory and anti-oxidant properties exhibited by Mp can be used in treating neuro-inflammatory circumstance in the case of PD [25].

Withania somnifera

Withania somnifera (Ws) (Ashwagandha) belonging to family Solanaceae, is usually known as "Indian Winter cherry" or "Indian Ginseng". It is used from decades as a Rasayana for its large range of health benefits and is one of the most important herbs of Ayurveda (the conventional system of medicine in India). Rasayana is a herbal preparation that promotes a condition of physical and mental health which helps in expanding happiness.

It is given to small children as tonics, and is also taken by the grown-up and elderly to increase longevity Ashwagandha holds the most renowned position among the ayurvedic rasayana herb. It is known as "Sattvic Kapha Rasayana" Herb [26]. Most of the Rasayana herbs are adaptogen / anti-stress agents in nature. Ashwagandha can be mixed with water, ghee (clarified butter) or honey as it is commonly available as a churna, a fine sieved powder. The function of the brain and nervous system can be enhanced and the memory can be improved by using Ashwagandha. It revamps the function of the reproductive system promoting a healthy sexual and reproductive steadiness. As it is a potent adaptogen, it enhances the body's pliability to stress. By, improving the cell-mediated immunity, it also enhances the body's defense against disease. Protection against cellular damage caused by free radicals can be overcome by the strong antioxidant properties of Ashwagandha [27].

Ws include certain biologically dynamic chemical constituents such as alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins [28]. Some of the anti-stress agents namely Sitoindosides and acylsterylglucosides are also present in Ashwagandha. Some of its active components, for example the sitoindosides VII–X and Withaferin-A, have been shown to have noteworthy anti-stress activity in opposition to acute models of experimental stress [29]. Immunomodulatory actions are supported by many of its constituents [30]. The aerial parts of Ws yield 5-dehydroxy withanolide-R and withasomniferin-A [31].

The traditional system of medicine, Ayurveda is practiced since 6000 BC [32]. Ashwagandha's root is regarded as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. The root smells like horse ("ashwa") that is why it is called Ashwagandha and moreover on consuming it gives the power of a horse. It is widely used in emaciation of children (when given with milk, it is the best tonic for children), debility from old age, rheumatism, vitiated conditions of vata, leucoderma, constipation, insomnia, nervous breakdown, goiter etc. [33]. Reduction the inflammation at the joints can be done forming the paste when roots are crushed with water [34]. It is also usually applied in carbuncles, ulcers and painful swellings [35]. In the case of snake venom as well as in scorpion-stinging, it is prescribed to take its root in combination with other drugs. It also helps in leucorrhoea, boils, pimples, flatulent colic, worms and piles [36]. Among all Ashwagandha varieties, the Nagori Ashwagandha is the ultimate one. It is much beneficial when fresh Ashwagandha powder is used [37]. The leaves are suggested in fever, painful swellings and are of bitter taste. The flowers are astringent, depurative, diuretic and aphrodisiac. The seeds being anthelmintic when combined with astringent and rock salt remove white spots from the cornea. Ashwagandharishta prepared from it is used in hysteria, anxiety, memory loss, syncope, etc. It also acts as a stimulant and increases the sperm count [26].

All the changes in antioxidant enzyme activities, catecholamine content, dopaminergic D2 receptor binding and tyrosine hydroxylase expression induced by 6- hydroxydopamine (6-OHDA) (an animal model of Parkinson's disease) in rats was prevented by pretreatment with Ashwaganda extract in a dose-dependent manner. Thus, these results propose that Ashwagandha may be supportive in protecting the neuronal injury in PD [15].

Ws are very holy herb of the Indian Ayurvedic system of medicine. It is especially used as a nervine tonic and for treating various kinds of disease processes. Ws exhibits strong anti-oxidant properties and possess wide range of active components including withaferin A, withanone and other flavonoids. Ws have been extensively studied for its anti-oxidative effect, synergistic effect with other medicinal herbs and its efficiency to increase catecholamines level and regulation of apoptotic processes.

Recent updates on Ws

Ahmad et al., have shown the Neuroprotective effects of Ws on 6-hydroxydopamine induced Parkinsonism in rats [38].

Oral treatment of Ws root extract resulted in a significant improvement in the mice's behavoiur and antioxidant status, along with a significant reduction in the level of lipid peroxidation in MPTP induced parkinsonian mice model [39].

Ws leaf extract protect oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease [40].

In 2009 RajaSankar et al., explore the anti-PD activity of Ws in MPTP induced parkinsonian mouse model and proved that it improves catecholamines and physiological abnormalities seen in a PD mouse [41].

Prakash et al., have shown the neuroprotective role of Ws root extract in Maneb–Paraquat induced mouse model of Parkinsonism [5]. In this published paper, Prakash et al., have justified the neuroprotective role of the Ws root extract against MB–PQ induced dopaminergic neurodegeneration, in PD mouse model. Ws extract inhibits the oxidative stress occurring in nigrostriatal tissues and concurrently increases the counts of TH positive cells in SN region of the MB–PQ induced PD mouse brain. Thus, the improvement in walking pattern seen in the Ws treated PD mouse model is principally due to up-regulation of TH expression in the SN region of the brain. Thus, from this study, it can be seen that Ws has strong antioxidant potential and its ROS scavenging property plays an important role in the avoidance of PD by fighting against neurodegeneration. Taken together, it can be said that Ws extract appears to be a potential drug candidate in the Parkinson's neuroprotection.

Parkinsonian phenotypes are alleviated using Ws by inhibiting apoptotic pathways in dopaminergic neurons [42]. In this study, Prakash et al., has shown that Ws causes augmentation in levels of catecholamines, improvement in motor activity, reduction in free radical generation and reduction of activated astrocytes in a PD mouse model. In addition, Ws have shown evidence of anti-apoptotic activity through the improvement of Bcl2 expression and turn down in level of Bax. Thus, the results here make clear the mechanism of action of Ws plant extract as a potent neuroprotectant.

In addition, Ws has shown neuroprotection of dopaminergic

neurons in substantia nigra pars compacta region of mid-brain in treatment of Parkinsonian mice model [43].

Standardized extract of Ws protect rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila melanogaster [44].

Anti-PD property of Ws is also explored in genetic model of PD in the paper entitled "Functional and Morphological Correlates in the Drosophila LRRK2 loss-of-function Model of Parkinson's disease: Drug Effects of Withania somnifera (Dunal) Administration" [45]. This paper demonstrates that methenolic root extract of Ws can be usefully employed to counteract some deficits associated with this condition.

Synergistic effect of *Mp* and *Ws*

Prakash et al., in 2013 have explored about the synergistic effect of Mp and Ws in paraquat induced Parkinsonian mouse model [6]. In this study, mice were administered i.p. injections of PQ (10 mg/kg body wt.) twice weekly for 9 weeks to induce PD. Then they treat PQ intoxicated mice individually with Mp (100 mg/kg) and Ws (100 mg/kg) and then synergistically (Mp-50 mg/kg+Ws-48 mg/kg). In combination (Mp+Ws), these herbal plants have shown the effective neuroprotective activity as compared to individual Mp and Ws treatment. Together, they successfully attenuate PQ induced neurotoxicity, which is evident from the improved level of TH activity in SN region of mice brain indicating rescued levels of dopamine. The behavioural and antioxidant recovery is also a significant indicator of the neuroprotective action of these herbal plants. This study gives strong substantiation for the beneficial effect of the co-administration of Mp+Ws on PD related symptoms in PQ induced Parkinsonian mice. The results of this nicely demonstrated paper suggest that Mp and Ws may offer a platform for future drug discoveries and novel treatment strategies for PD treatment.

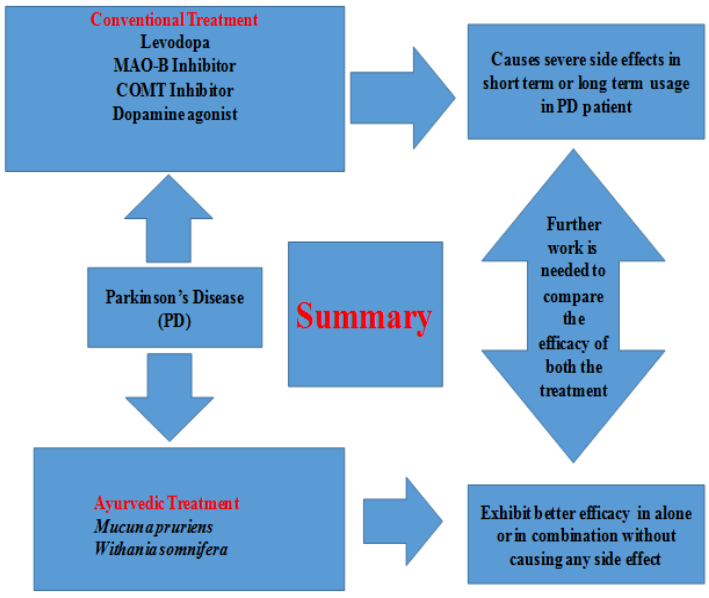
Summary

Concluding remark

In the case of PD, a holistic approach is provided by the Ayurveda. Levodopa is the only gold standard medication used since long time but it causes several side effect like drug induced dyskinesia. Natural compound and herbal extract like Mp and Ws shows strong and potential neuroprotective activity in chemical induced PD mice model. In addition the synergistic effect of Mp and Ws show effective neuroprotective activity. A lot of clinical trial has been done on herbal extract and their isolated compound in order to patent the drug. Thus a new scenario has been opened in the area of PD by using ayurvedic and herbal plant and their extract.

Current & future developments

Until now, Clinicians and researchers working in the field of PD therapy have identified that the existing drugs do not offer optimal and long lasting therapeutic benefits to patients. This can be said by the fact that most predictable PD drugs elicit adverse effects following chronic exposure. The fundamental apprehension also has been the inability to check the continued degeneration of the dopaminergic neurons. The current review highlights that natural products including herbal extracts like Mp and Ws offer promising neuroprotective properties, target multiple pathways, are least toxic in humans and could be administered with dopamine replenishing drugs as adjunctive therapy. Although most of the products look promising, a inclusive initiative to carry out wide-ranging clinical examination is required since many of the naturally occurring drug compositions have to be adapted to pharmacological appliance for successful neuroprotection and alleviation of dyskinesias.



Figure

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