DRUG DESIGN AND THERAPEUTIC STUDIES OF NATURAL PRODUCTS USED IN THE MANAGEMENT OF PARKINSON’S DISEASE (PD)

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OUTLINE

- Drug Development processes
- Animal and Cellular models of Parkinson’s disease (PD)
- Overview of Parkinson’s disease
  - Diagnosis
  - Treatment
- Natural products used in the management of PD
- Conclusion
Drug Development processes
Drug Development Process

- incorporates the entire process of finding, developing and clinical testing of novel drug candidates.

- Involves these 2 main stages:
  - Preclinical research
    - *in vitro/in vivo* toxicity studies
    - ADME
    - Mutagenicity studies among others
  - Clinical development- Phases I, II, III clinical trials.
Drug design

- is the method of finding drugs by a pattern, based on their biological targets. It is a sub-section of drug development.

- The **primary goal** is to find, develop and market new chemical entities that can be used to treat diseases that may have superior properties when compared to currently available drugs.
How are drugs discovered? – Drug discovery can be achieved without a Lead or through Lead

How are they developed? – Lead modification and optimisation, screening, safety evaluation & formulations research.
Drug Discovery?

- Development from herbal or traditional medicines (may be from plants, animals, marine organisms etc)

- Lead compounds need to be identified by isolation of active ingredients of folklore/traditional medicines
  - This may involve identification of candidate’s synthesis, characterization, screening and assay for therapeutical efficacy
Clinical trials

- The drug is tested for efficacy against disease and human toxicity. Appropriate dosage limits are explored as well as the fate of the drug in the body.

- **Phase I** – Involve a small group (20 to 100) of healthy volunteers to discover if the drug is safe in humans (few months to 18 months).

- **Phase II** – Involve 100 to 500 patients who actually have the disease being studied to see if the drug works by the expected mechanism and actually produces measurable results (1 -3 yrs).

- **Phase III** – Involve thousands of patients to generate statistically significant data about safety, efficacy, and an overall benefit/risk profile (2 - 6 yrs).

- **Phase IV** – post market surveillance to monitor drugs efficiency, locate reports of adverse effects and assess relative efficacy.
New drug application

- Once clinical trials are complete and the compound is shown to be safe and effective in humans, researchers can submit a New Drug Application (NDA) to the regulatory agency.

- If the regulatory agency approves the NDA, the medicine can be marketed and sold to consumers.

- If the clinical trial shows that the drug is unsafe or ineffective, the drug cannot be marketed.
Individual Laboratory Contributions to Drug Discovery and Development

- Target identification – new receptor, enzyme, pathway, protein, other.
- Target validation – data linking target to human disease
- Finding new molecule – chemical or biological
- Screening assays – cell lines, animal models, others.
- Data on drug-like characteristics – PK, toxicology
- Development tools – PD Biomarkers including biochemical assays, PET ligands, electrophysiological measures, others.
- Efficacy measures – clinical scales, cognitive tests, functional measures, self reported outcomes, electronic health recording
- Technologies to improve efficiency of trial completion – recruiting technologies, electronic data capture and tracking, trial simulation, safety monitoring, other.
Animal and Cellular models of Parkinson’s disease
PD Models

- Generally, different models of PD can help us to develop agents that can be useful as anti-PD agent and provide equitable evidence for the conduct of clinical trials.
Animal and Cellular Models of PD

- There are several animal and cellular models for PD that are extensively used.
- **Animal models** are used to obtain information on the neuroanatomical and behavioral aspects of the disease while the **cellular models** are for analyzing the mechanism of cell death including the molecular bases of the affliction.

- The best-characterized animal model of PD has been developed by using the
  - neurotoxin 6-hydroxydopamine (6-OHDA) was the first agent
  - neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
- The 6-OHDA model of the PD is based on the generation of free radicals as well as potent reversible inhibition of mitochondrial electron transport chain (ETC) complex-I and -IV
Animal models of PD

- 6-OHDA induced (12, 16 or 20 μg, s.i.),
- MPTP induced (30, 40, or 60 mg/kg, i.p.),
- Buthionine sulfoximine induced (3 mmol/kg, i.p.),
- Homocysteine induced (2 mol/l, i.c.v.)
- Lipidosome induced (1 μg/ml, s.i.),
- Acute Cu-intoxication (10 mg/kg, i.p.)
- Rotenone-induced,
- Paraquat-induced rats
- Acrolein-induced
- H$_2$O$_2$-induced neurotoxicity

MPTP 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine
6-OHDA 6-hydroxy dopamine;
The cell culture techniques allow replicating few of the characteristics, by deriving patient-specific cell lines from individuals with sporadic forms of PD and also those with known disease-causing mutations.
There are cell lines with neuronal lineage, which have the potential to serve as a human cellular model for PD when differentiated into DA-ergic neurons.

The cell culture models have advantages over animal models since they can be human genome based, and thereby allow the direct investigation of pathophysiological characteristics in far less time, less labor intensive and such techniques can be developed for high throughput screening of therapeutic compounds.

- 6-ODHA-induced neurotoxicity in SH-SY5Y cells
- MPTP-induced neurotoxicity in mouse and glutamate-induced excitotoxicity in primary cortical neuron cultures
Research methods

- Immunohistochemistry (IHC)
- HPLC analysis
- Western blot analysis
- Reverse transcription-polymerase chain reaction (RT-PCR)

Behavioral tests

- Immunofluorescence (IFC)
- Elisa the enzyme-linked immunosorbent assay (ELISA)
- Luciferase assay
- Neurochemical Analysis
ANIMALS

- Sprague-Dawley rats
- Wister rats
- C57BL/6 mice
- Swiss albino mice
- ICR strain mice

To some extent, animal experiments may give insights into the mechanisms of PD, but a single study can not fully uncover all the details.
Overview of Parkinson’s disease
Overview

- PD was first described by the English Physician James Parkinson in 1817.

- It is the second most common human neurodegenerative disorder.

- The etiology of PD is mainly related to genetic and environmental factors, as well as mutual interactions and epigenetic factors influences.
Overview

- The neuropathological symptoms of PD include the degeneration of dopaminergic neurons in the substantia nigra pars compacta, and presence of intracellular inclusions of \( \alpha \)-synuclein (\( \alpha \)-syn) aggregates.
Overview cont’d

- Symptoms caused by insufficient dopamine.

- 3 main symptoms:
  - Tremors
  - Rigidity
  - Slowed motion (Bradykinesia)

- Other symptoms include:
  - Dementia, sleep disturbances, depression, etc.
Overview cont’d

- Common cause of chronic progressive parkinsonism.

- PD is typically idiopathic, but it could be caused by
  - infection,
  - drugs,
  - trauma,
  - Toxins
  - Gene mutation
Pesticides are environmental toxins which cause degeneration of the dopamine producing cells. It is a well known fact that the risk for PD is elevated by the herbicide parquet.

oxidative stress is a key driver of the complex degenerating cascade underlying dopaminergic neurodegeneration in all forms of PD (Blesa et al., 2015).
MANAGEMENT

The aims of management of PD are to:
- provide general support
- reduce symptoms
- prevent further degeneration
- induce reversal or regeneration
Most of the current pharmacotherapeutic approaches in PD are aimed at replenishing the striatal dopamine. Although these drugs provide symptomatic relief during early PD, many patients develop motor complications with long-term treatment.

Further, PD medications do not effectively tackle tremor, postural instability and cognitive deficits. Most importantly, most of these drugs do not exhibit neuroprotective effects in patients.

Consequently, novel therapies involving natural products/molecules with neuroprotective properties are being exploited for adjunctive therapy.
Hence:

- Researchers are concentrating on the development of innovative neuroprotective molecules of natural origin with high efficacy and low side effects to prevent neuronal deaths in Parkinson's disease.
Justification for Natural Products in the Management of Parkinson’s Disease
Many natural products have been reported to possess anti-PD properties as a result of not only their well-recognized antioxidative and anti-inflammatory activities but also their inhibitory roles regarding protein misfolding and the regulatory effects of PD related pathways.

Natural products are a promising choice for PD treatment and disease modification (Solayman et al., 2017) as increasing number of monomer components of natural products have shown anti-PD effects in PD cellular and animal models.
Phytocompounds have been identified as promising target of research in the quest for new pharmaceutical compounds as they can produce secondary metabolites with novel chemical structure.

Some natural products have been found to be involved in regulating gene activity and/or protein density in PD as a neuroprotective agent.
Several natural anti-parkinsonian therapeutics show promise in providing neuroprotective therapy against this devastating disorder in preclinical animal studies.
In the past few years, there is an enormous emphasis on the medicinal use of plant extracts, which are also reputed for their therapeutic claims in a variety of traditional medicines.

The traditional preparations usually contain many medicinal plant extracts that have been shown better therapeutic efficacy on neurological diseases.
Natural products used in the management of Parkinson’s Disease
Curcumin is a polyphenol and an active component of turmeric (Curcuma longa) and it has been reported to be involved in the prevention of heavy metal related Parkinsonism (Abbaoui et al., 2017).

- offers neuroprotection in animal models of PD probably via:
  - antioxidant capabilities,
  - anti-inflammatory and
  - anti-apoptosis.
Uncaria rhynchophylla

Curcuma longa

Mucuna pruriens

Pueraria lobata

Scutellaria baicalensis Georgi (Labiatae)

Withania somnifera

Uncaria rhynchophylla
L-DOPA is naturally found in beans, especially Mucuna spp, which has been proven experimentally to enhance DA levels in the brain. The seed of *Mucuna pruriens* is an existing medication therapy for PD.

Puerarin is the major bioactive ingredient isolated from the root of the *Pueraria lobata* (Willd.), studies showed that it displayed protective effects on rotenone-based cell and animal models for PD. The potential mechanism has been suggested to be via antioxidative stress, anti-apoptosis and the degradation of aggregated proteins enhancement.
Baicalein (5,6,7-trihydroxyflavone) is an important flavonoid compound mainly isolated from the roots of *Scutellaria baicalensis* Georgi (Labiatae).

Many studies have clearly demonstrated that baicalein protected 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenylpyridinium (MPP+), glutamate, amyloid-β (Aβ), hydrogen peroxide (H₂O₂), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and methamphetamine-induced neurotoxicity in animal models and cell lines.

Baicalein as a potential molecule of natural origin to target α-syn as it inhibits aggregation of α-syn protein.
Li et al. (2017) also reported the therapeutic properties of baicalein against PD.

- **Baicalein** was observed to halt PD progression by reducing oxidative stress, inhibiting excitotoxicity, inhibiting aggregation of disease-specific amyloid proteins, and stimulating neurogenesis and antiapoptosis as well as anti-inflammatory properties.

It was concluded from all the studies that baicalein has potential therapeutic effects in Parkinson’s disease.
**Withania somnifera (Solanecea)**

- *Withania somnifera*
  - reduced oxidative stress and increased catecholamine content in 6-hydroxydopamine- (6-OHDA-) induced rats,
  - restored the antioxidant status, and reduced lipid peroxidation as well as increase striatal catecholamine contents in 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine- (MPTP-) induced mice.

- Ethanol extract of *W. somnifera* also attenuated the increase of acetylcholinesterase activity and restored dopamine level and mitochondrial electron transport chain enzyme complex activities.

- Hence, these findings suggested that *W. somnifera* extracts has antiparkinson’s disease activity and can be used therapeutically.
Ginseng

- Ginseng is a perennial herb belonging to the *Panax* genus of the Araliaceae family.

- In an animal model of PD, Korean red ginseng improved the behavioral impairment of mice in the pole test, inhibited dopaminergic neuronal death, and decreased cyclin-dependent kinase 5 (Cdk5) and p25 expressions as well as increase p35 expression in the substantia nigra and striatum of MPTP-induced mice.
Uncaria rhynchophylla

- It reduced neuronal cell death and ROS generation, restored the GSH level, and prevented the caspase-3 activity in 6-OHDA-induced toxicity in PC12 cells as well as reduced neuronal loss in dopaminergic neurons in the substantia nigra in 6-OHDA-induced rats.

- It exhibited anti-Parkinson’s activity in 6-OHDA rat model.

- It is reported to increase cell viability, attenuate dopaminergic neuronal loss of substantia nigra and striatum in MPP+-induced SH-SY5Y cells and
Uncaria rhynchophylla

- It was established that U. rhynchophylla has neuroprotective action in protecting neuronal damage through multiple pathways which could be due to the beneficial effect of active compounds as well as the combination effect of those compounds present in the plant.

- Neuroprotective effect of alkaloid such as hirsutine, rhynchophylline, and isorhynchophylline in Uncaria rhynchophylla have been reported.
Investigation on neuroprotective effect of several seaweeds in 6-OHDA-induced toxicity in SH-SY5Y cells showed that seaweed extracts had increased the cell viability, reduced oxidative stress, protected mitochondrial membrane potential, and reduced caspase-3 activity.
The phytochemicals targeting α-synuclein in the *in vitro* models of Parkinson’s disease (Javed et al., 2019).

<table>
<thead>
<tr>
<th>Phytochemicals (Plant name, family)</th>
<th>In vitro model system</th>
<th>Effects and mechanisms observed</th>
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</thead>
<tbody>
<tr>
<td>3a-Acetoxyeudesma-1,4(15),11(13)-trien-12,6a-Olide (<em>Laurus nobilis</em>, Lauraceae)</td>
<td>Dopamine-induction and α-syn formation in neuroblastoma cells (SH-SY5Y)</td>
<td>Inhibits apoptosis by decreasing of caspase-3 and p53 activation and increasing Bcl-2 Suppresses tyrosinase activity and ROS generation; Suppresses quinoprotein and α-syn formation</td>
</tr>
<tr>
<td><strong>Baicalein</strong> (<em>Scutellaria baicalensis</em>, Lamiaceae)</td>
<td>α-syn aggregation assay</td>
<td>Inhibits the formation of α-syn fibrils Disaggregates α-syn fibrils involving Tyr</td>
</tr>
<tr>
<td><strong>Curcumin</strong> (<em>Curcuma longa</em>, Zingiberaceae)</td>
<td>α-syn in genetic synucleinopathy mouse line overexpresses wild-type α-syn</td>
<td>Improves gait impairments Increases phosphorylated α-syn in presynaptic terminals without affecting α-syn aggregation</td>
</tr>
<tr>
<td>Phytochemicals</td>
<td>Activity</td>
<td>Effects</td>
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<td>Crocin-1,2, safranal and crocetin, and its analogs; hexadecanedioic acid, norbixin, and trans-muconic Acid (<em>Crocus sativus</em> L., Iridaceae)</td>
<td>α-syn aggregation and fibril dissociation assays</td>
<td>Prevent dissociation of fibrils and inhibit α-syn aggregation. Crocetin appears most potent and thereafter norbixin.</td>
</tr>
<tr>
<td>Thymoquinone (<em>Nigella sativa</em>, Ranunculaceae)</td>
<td>α-syn-induced synaptic toxicity in rat hippocampal cells and human induced pluripotent stem cell (iPSC)-derived neurons</td>
<td>Reduces the α-syn-induced loss of synaptophysin. Enhances synaptic vesicles recycling in the presence of α-syn. Protects iPSC-derived neurons and maintain firing activity.</td>
</tr>
<tr>
<td>Ginsenosides (Rb1) (<em>Panax ginseng</em>, Araliaceae)</td>
<td>α-syn aggregation and toxicity using biophysical, biochemical and cell-culture techniques</td>
<td>Inhibits α-syn fibrillation and disaggregate preformed fibrils and inhibit the seeded polymerization of α-syn.</td>
</tr>
</tbody>
</table>
Figure 1. The effects of phytochemical-rich plants in counteracting the cascade (plain black arrows) of molecular events, which occur in synucleinopathies and Parkinson’s disease (PD). These include (i) oxidative stress and accumulation of Reactive Oxygen Species (ROS) arising from altered dopamine (DA) metabolism (DA oxidation), (ii) endoplasmic reticulum (ER) and mitochondrial stress, (iii) structural alterations of -syn, formation of insoluble aggregates up to Lewy bodies where native -syn monomers are sequestered (dashed black arrows), (iv) neuroinflammation, and (v) autophagy impairment due to either altered autophagosome biogenesis or impaired fusion between lysosomes and autophagosomes (dashed black arrows). The buildup of ubiquitinated -syn aggregates contributes to further impairing the autophagy machinery thus fueling a vicious circle where damaged autophagy substrates accumulate due to impaired clearance and turnover. This, in turn, contributes to increasing the overall vulnerability of DA neurons and promoting the spreading of -syn (dashed black arrows). Phytochemicals from the plants represented here confer neuroprotection by preventing or reverting (blue arrows) this pathological cascade, starting from autophagy induction to inhibition of -syn aggregation, neuroinflammation, and oxidative stress. (Limanaqi et al., 2019)
Mechanisms of action of Natural Products are related to their neuroprotective effects that are based on:

- anti-oxidant effect that can guard substantia nigra (SN) neurons and increase striatal dopamine count and chelate Fe$^{2+}$ in the 6-OHDA model.
- anti-apoptosis activity
- mitochondrial protection in various PD models.
• Restoration of mitochondrial membrane potential,

• Increase in Cu/Zn SOD (Superoxide Dismutase)

• Restoration of cell viability especially in 6-OHDA-lesioned MES (mouse embryonic stem) 23.5 cells

• increase in striatal dopamine and DOPAC (3,4-Dihydroxyphenylacetic acid) levels in injected mice
Improvement on the neurobehavioral function.

Restoration of the levels of tyrosine hydroxylase, Dopamine, DOPAC (3,4-Dihydroxyphenylacetic acid).
SUMMARY

- Plant-derived natural products have the potential to be used as drugs for the treatment of PD.

- The principal modes of neuroprotection are antioxidant property, prevention of apoptosis, inhibition of DA-transporter function, prevention of microglial activation, anti-inflammation, decrease in nitric oxide synthesis, monoamine oxidase inhibition, and enhancement of trophic factors, including BDNF and GDNF.
Conclusion

- Some of these natural compounds have potentials in the management of PD. Hence, these compounds can lay the foundation for a new therapeutic approach for the treatment of PD.

- Natural compounds are easily accepted by patients because they are considered healthier and safe than the synthetic drugs.
References


