Introduction:

Parkinson’s disease is a multifactorial progressive neurodegenerative disorder. Disease develops gradually, may be sometimes starting with a barely noticeable tremor in just one hand. But it is said that tremor is the most well-known sign of Parkinson's disease, and other symptoms due to the neuro-degeneration may be the stiffness or slowing of movement (bradykinesia), rigid muscles, impaired posture and balance, loss of automatic movements and speech or writing changes. The symptoms of this disorder may be characterized by selective degeneration of dopamine neuron cells in the substantia nigra pars compacta and intra-neuronal accumulation of misfolded, fibrilar alpha-synuclein protein in the Lewy bodies.

Dopamine deficiency is one of the main reason for the movemental disorder. Dopamine is an organic chemical belonging to the catecholamine and phenethylamine families. It is produced in two different areas of brain, substantia nigra and ventral tegmental area. It functions as a neurotransmitter regulating the movements of the body and also plays several other important functions in the brain as well as body. Dopamine which is produced from substantia nigra helps in beginning movement and speech. So, when dopamine in this region start to die off, it results in having trouble initiating the movement and this is considered as one of the major symptoms of the Parkinson’s disease.

Alpha-synuclein is a protein consisting of 140 amino acids, which is coded by SNCA gene and this protein is abundantly present in the human brain cytosol, usually at the tip of nerve cells which are known as neurons. Alpha-synuclein is a soluble monomer and natively unfolded. The exact role of this protein is poorly understood but it is thought to regulate the release of dopamine which is involved in controlling voluntary and involuntary movements.

Single nucleotide polymorphism of SNCA gene i.e SNCA missense mutations accelerates alpha-synuclein fibril formation implicating protein misfolding and aggregations of this protein into Lewy bodies in Parkinson’s disease. The exact cause for the mutation is not yet reported but the misfolding is due to the structural or conformational changes in alpha-synuclein. Further, if the mutated alpha-synuclein protein undergoes post translational modification, it results into pathological processes like protein aggregation or Lewy body formation in the brain region. Post translational modifications of alpha-synuclein like phosphorylation leads to aggregation of alpha-synuclein, ubiquitination may cause change in activity or functions of the protein and nitration causes increase in the level of neurotoxic α-synuclein species and finally the cell death. The structural or conformational changes may be due to the binding and interacting of protein to lipid surfaces in the brain region. These interactions is said to induce change in the alpha-synuclein structure from unfolded to folded secondary helical structure. It may form dimers or oligomers and these oligomers are said to
be highly toxic to the brain cells. It is mentioned in some of the literatures that misfolding of alpha-synuclein may be the reason for death of dopaminergic neuron cells which finally results in the movemental disorders which is one of the important symptoms of parkinson’s disease.

**Therapeutic Treatments:**
- **I-dopa (levodopa)** – first line treatment
- **Anti-aggregating molecules** (Curcumin, a dietary polyphenol, it’s derivatives zingerone and their biphenyl analogues).
- **Ceftriaxone** as a therapeutic agent which blocks the alpha-synuclein aggregations (under clinical trials).

**Objectives:**
- Regeneration, elicitation of bioactive compounds in callus extracted from *Mucuna pruriens* endangered medicinal plant.
- Screening and characterization of bioactive compounds.
- Pre-clinical investigation of anti-parkinson’s activity of the bioactive compound using biomolecular simulation studies.
- Pharmacological evaluation of bioactive compound on animal models.

**Materials and methodology:**

I. MATERIALS
*In-vitro* regeneration of *Mucuna pruriens* and extraction of bioactive principles from callus.

i) **MEDICINAL PLANT:**
- *Mucuna pruriens* L (DC)

ii) **CHEMICALS:**

- **Plant tissue culture:** Macro-nutrients, Micro-nutrients, Vitamins, Minerals, Growth hormones, Charcoal, Sucrose, Agar, Distilled water.

- **Explant Sterilization:** 5% v/v teepol, Distilled water, Ethyl-alcohol, 0.1% HgCl2, 5-10% Sodium hypochlorite solution, De-ionized water.

- **Solvents for Extraction:** Distilled water, Methanol,

II. METHODS:

i) **PROCUREMENT, SOWING OF THE SEEDS, INOCULATION, INCUBATION AND EXTRACTION:**

*Mucuna pruriens* is an endangered medicinal plant belonging to the Fabacea family. The seeds of this plant contain 4-5% I-dopa which is used for treating neurodegenerative disorder. So, our first objective was to regenerate the endangered plant. The *mucuna pruriens* seeds were procured from Himalayan drugs, Bangalore. Due to the hardness of the seeds, they were soaked in water for three days and then were sowed in college campus under suitable environmental conditions. For *in-vitro* regeneration, three week old plantlet was choosen. The selected explants were inoculated onto the prepared M.S media and incubated at 20±5°C for about 30 days. The methanol extraction was performed to extract the crude extract of *Mucuna pruriens* and further this extraction was used for pre-clinical trials.
ii) VIRTUAL SCREENING AND OPTIMISATION STUDIES (BIOSIMULATION STUDIES):

Step 1: The homology study of wild alpha-synuclein was carried out by identifying homologous alpha-synuclein.
Step 2: Among the homologous alpha-synuclein, only human mutant variants have been selected for the further studies.
Step 3: Further selected homologous alpha-synuclein were selected for the study of variation among mutants and wild type.
Step 5: Mutations were identified by performing Multiple Sequence Alignment using clustal-omega.
Step 6. After identifying the possible mutations among the mutant variants, wild alpha-synuclein was selected for docking with ceftriaxone.
Step 7: Molecular docking was performed using AutoDock 4 tool to get the optimized Ceftriaxone.

iii) PRE-CLINICAL STUDIES ON MOUSE MODELS:

Parkinson’s disease (PD) is characterized by severe locomotor deficits and is commonly treated with the dopamine (DA) precursor L-3,4-dihydroxyphenylalanine (L-DOPA), but its prolonged use causes dyskinesias referred to as L-DOPA–induced dyskinesia (LIDs). In our present studies for pre-clinical trials, we used animal models of PD, targeting β-arrestins in PD. L-DOPA therapy might prove to be a desirable approach. Here we show in a bilateral DA-depletion mouse model of Parkinson’s symptoms that genetic deletion of β-arrestin2 significantly limits the beneficial locomotor effects while markedly enhancing the dyskinesia-like effects of acute or chronic L-DOPA treatment. Viral rescue or overexpression of β-arrestin2 in knockout or control mice either reverses or protects against LIDs and its key biochemical markers. In other more conventional animal models of DA neuron loss and PD, such as 6-hydroxydopamine–treated mice or rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–treated nonhuman primates, β-arrestin2 overexpression significantly reduced dyskinesias while maintaining the therapeutic effect of L-DOPA.

Results and discussions:

i) In-Vitro Regeneration of *Mucuna pruriens* And Extraction of Bioactive Principles from Callus.

![Fig 1: Growth of plant after 4 weeks](image)

Fig 1: Growth of plant after 4 weeks
ii) Extraction of bioactive principles from callus of inoculated *Mucuna pruriens* explants.

![Mucuna leaf extract dissolved in Methanol](image1)

![Vacum dried Mucuna seed extract](image2)

iii) Virtual Screening of Ceftriaxone and Optimisation of Ceftriaxone against Alpha-Synuclein for Anti-Agglutinin Activity:

![Results of Multiple Sequence Alignment](image3)

iv) AutoDock Results:

![Optimized Ceftriaxone](image4)
v) PRE-CLINICAL STUDY RESULTS:
Designed Combo drug containing L-DOPA and Ceftriaxone, is administered to DAT-KO knockout mice models and are still under pre-clinical observations. Pre-clinical studies may proceed for one more week and results will be interpreted and will be discussed during the evaluation process.

SCOPE FOR FUTURE WORK:
While there’s is no cure for Parkinson’s disease, our present studies of developing the combo drug may lead to the improved treatments. As many of the scientists and doctors are working together to find a treatment which can cure the disease or prevention techniques, so designed combo drug may have a future scope in treating the Parkinson’s disease. Aside from other therapies as mentioned above, combo drug could help stop the progression of the disease and prevent symptoms from getting worse. In this way our present work provides idea for the scientists or any other person working towards the Parkinson’s disease to find out new ways to control the progression of disease in much faster way.

References:
6. Lucia Raffaella.; Lampariello; Alessio Cortelazzo; Roberto Guerranti; Claudia Sticozzi; Giuseppe Valacchi; (2012) The Magic Velvet Bean of Mucuna pruriens. Vol2(4) [PMC]