2. LITERATURE SURVEY

2.1. Parkinson’s disease:

2.1.1. Introduction of Parkinson’s disease:

Parkinson’s disease is a progressive neurodegenerative movement disorder that is estimated to affect approximately 1% of the population older than 65 years of age\textsuperscript{46,47}. Clinically the cardinal symptoms were first described by James Parkinson 1817 with most patients presenting bradikinesia, resting tremor, rigidity, and postural instability\textsuperscript{48}. A number of patients also suffer from autonomic, cognitive, and psychiatric disturbances\textsuperscript{49}. The major symptoms of PD result from the profound and selective loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc)\textsuperscript{50}. The pathological hallmarks of PD are lewy bodies (LBs) and dystrophic neurites (Lewy neurites) present in surviving neurons \textsuperscript{51}.

2.1.2. Incidence and Prevalence of Parkinson’s disease:

PD is a common neurodegenerative disorder, characterized by neuronal cell loss in the substantia nigra (SN) and subsequently reduced secretion of dopamine (DA). PD is the second most common neurodegenerative disease after Alzheimer's disease, affecting up to 1% of the elderly population over the age of 65. Epidemiological studies have estimated a cumulative prevalence of PD of greater than one per thousand with the prevalence being limited to senior populations; this proportion increases nearly 10-fold. A family history of risk factors is a key factor for PD development. Indeed, the estimated genetic risk ratio for PD is approximately 1.7 (70%
increased risk for PD if a sibling has PD) for all ages, and increases over 7-fold for those under 66 years of age\textsuperscript{52, 53, 54}. The age-adjusted prevalence rate of PD revealed from a pilot study of at least a 42.5% increase in the disease compared to 1966\textsuperscript{55}. The role for genes contributing to the risk of PD is also significant. Recently, monogenically PD as familial PD (FPD) has been identified and seven causative genes for FPD have been identified so far. Thus, the role of genetic factors and also increasing age play an important risk factor for the development of PD.

2.1.3. Clinical Characteristics of PD:

Resting tremor at a frequency of 4-6Hz occurs at rest but decreases with voluntary movement in about 70% of Parkinson’s patients. Rigidity refers to increased resistance (stiffness) initially occur only to trunk region but also extends to passive movement of limbs. Bradykinesia (slowness of movement), manifest as a variety amplitude, and akinesia (absence of normal facial expression, drooling, microphagia, and decreased stride length during walking. PD patients also develop stooped posture and possibly lose postural reflexes. Freezing of gait, the inability to begin a voluntary movement such as walking is a common symptom of Parkinsonism. Impairment in different cognitive domains such as executive functions, memory, and visuospatial skills occurs frequently in PD even in the early stages of the disease\textsuperscript{56}.

2.1.4. Neurochemical and Neuropathological Features of PD:

Cell loss in PD:
Idiopathic Parkinsonism (IPD) is characterized by progressive and profound loss of neuromelanin containing DAergic neurons in the substantial nigra pars compacta (SNpc). Moreover, slight gliosis and neuronal loss in the locus coeruleus, dorsal vagal nucleus with variable involvement of the nucleus basalis Meynert, and other sub cortical nuclei have been reported. Degeneration of pigmented neuronal systems located in the brain stem, particularly in SNpc, is the most striking pathological feature of PD. This causes striatal DA deficiency and all the major motor PD symptoms.

Neuropathological studies of PD-related neurodegeneration reveal DAergic neuronal loss to have a characteristic topology, which is distinct from the pattern seen in the aging process. In PD, cell loss is concentrated in ventrolateral and caudal portions of SNpc, where as during normal aging the dorsomedial aspect of SNpc is affected. The degree of terminal loss in the striatum (ST) appears to be more pronounced than that of SNpc DAergic neuronal loss, suffesting that ST DAergic nerve terminals are the primary target of the degenerative process. The neuropathology of PD is characterized solely by DAergic neuron loss which correlates with the progressive motor decline. However the neurodegeneration extends well beyond DAergic neurons, with many “non-DAergic” PD features. Neurodegeneration and Lewy bodies (LB) formation are found in noradrenergic (locus coeruleus), serotonergic (raphe), and cholinergic (nucleus basalis of Meynert, dorsal motor nucleus of vagus) systems, as well as in the cerebral cortex (especially cingulated and entorhinal cortices), olfactory bulb, and autonomic nervous system. Degeneration of hippocampal structures and cholinergic cortical inputs contribute to high rate of dementia that accompanies PD, particularly
in older patients. The clinical correlation to lesions in serotonergic and noradrenergic pathways is not clearly characterized as DAergic systems.

**Lewy bodies in PD:**

Apart from the loss of melanized nigrostriatal DAergic (DAergic) neurons another major pathological hallmark of PD includes the presence of intraneuronal proteinacious cytoplasmic inclusions “Lewy Bodies” (LBs). This result in the classical neuropathological finding of SNpc depigmentation where the SNpc DAergic cell bodies project primarily to the putamen. However LBs are not specific for PD as they are also found in Alzheimer’s disease (AD), other α-synucleinopathies such as dementia with LB disease (DLBD), and as “an incidental” pathologic finding in people of advanced age than the prevalence of PD. The role of LB in neuronal cell death is controversial, as the reasons for their increased frequency in AD and the relationship of incidental LB to the occurrence of PD. LBs are more than 15µm in diameter with an organized structure containing a dense hyaline core surrounded by a clear halo. Electron microscopy reveals a dense hyaline core surrounded by a clear halo. Electron microscopy reveals a dense granulovesicular core surrounded by radiating 8-10 nm fibrils.

2.1.5. **Etiology of PD:**

**Environmental factors:**

The specific etiology of PD is not known. It has been proposed to be multifactorial because of its sporadic nature. Epidemiological studies show that a number of factors may increase the risk of developing PD. These include the exposure to well water, pesticides, herbicide, industrial chemicals, farming and living in rural environment. A number of
Exogenous toxins have been associated with the development of Parkinsonism, including trace metals, cyanide, carbon monoxide, and carbon disulfide. In addition, the possible role of endogenous toxins such as tetrahydro-isoquinolines and beta-carbolines has also been implicated.

Human exposure to chemical compounds of synthetic origin, including pesticides, herbicides and insecticides has been the focus of several epidemiologic studies since the first description of Parkinson-like symptoms among individuals who had taken drugs contaminated with 1-methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP). MPTP is converted to 1-methyl phenylpyridinium (MPP⁺) by MAO-B in glial cells, and subsequently selectively concentrated in mitochondria of DAergic neurons where it interact with elements of the mitochondrial respiratory chain, leading to ATP depletion, and eventually cell death of the DAergic neurons. Paraquat is an herbicide structurally similar to MPTP; human exposure to paraquat has also been associated with an increased risk of PD, and studies on animal models demonstrate that paraquat induces a selective loss of DAergic neurons.

Human activities related to a possible pesticide exposure, including farming, living in rural areas and drinking water, are among risk factors associated with PD, where as smoking and drinking coffee are protective factors. A chronic occupational exposure to manganese is the cause of manganism, a condition characterized by tremor, rigidity and psychosis due to the accumulation of the metal in the basal ganglia. Although no changes in manganese brain concentration has been observed in PD, exposure to manganese has been linked to the risk of PD in some epidemiological studies. Copper exposure has been associated with PD, where as iron...
exposure alone was not; however exposure to combinations of iron and lead, iron and manganese and iron and copper was associated with PD. Controversial or negative results have been obtained for exposure to zinc, mercury and aluminium\textsuperscript{75,76,77}. Inflammation of the brain in early life as a consequence of head injuries, viral or bacterial or exposure to neurotoxicants, has been indicated as a possible contributor to the development of PD later in life\textsuperscript{62}, moreover it has been suggested that occupational exposure to viral (or other) respiratory infections might be one of the risk factors for the observed increased incidence of PD cases among teachers and healthcare workers\textsuperscript{78}.

**Genetic factors:**

The first gene discovered and mostly studied is the PARK-1 gene which encodes the alpha synuclein protein\textsuperscript{79,80}, and is present in PD and dementia with LBs (DLB) but not in normal human brains. The accumulation of \( \alpha \)-syn intracellularly at abnormally high quantities, in an aggregated form with in the neurons and sometimes glial cells, has been shown to contribute to the pathology of PD and also to generate hydroxyl radicals (OH\(^{\cdot}\)) upon the addition of the iron (Fe\(^{2+}\)). The identification of responsible mutations in certain genes, particularly alpha-synuclein, parkin, PINK1, DJ-1 and LRRK2, has increased our understanding of the clinical and pathological changes underlying Parkinson’s disease, with implications for patient diagnosis, management and future research.

**Ageing and PD development:**
Age is an important factor in PD expression. After the age of 30-40, the incidence increases with the age reaching a maximum at the 8th decade with a decline in prevalence thereafter. The progression of the aged in the total population is also expected to rise from 12.25 in year 2002 to 15.55 in the year 2020. Ageing in individuals is affected to a great extent by genetic factors, diet, social conditions, and the occurrence of the age related diseases. There is evidence that age induced alterations in cells are an important component of the aging of the organism. A number of cell functions decline progressively with age, for example oxidative phosphorylation in the mitochondria, the synthesis of the nucleic acids and structural enzymatic proteins are reduced. Oxidative damage to mitochondrial DNA increases with ageing and can induce point mutations thus, contributing to mitochondrial dysfunction and neuronal loss. Biochemical analysis revealed a shift in the extractability of parkin upon aging in humans but not in mice thus there is an effect of aging upon parkin in humans.

2.1.6. Mechanisms of Parkinsonian Cell Death:

**Oxidative stress (OS):**

OS and the consequent cell death in SNpc usually results from either an increased DA turnover, resulting in excess peroxide formation, a deficiency in glutathione (GSH), thus diminishing the brains capacity to clear H₂O₂ or an increase in reactive iron thereby, promoting OH⁻ formation. Most of evidence suggesting OS is linked to the pathogenesis of PD comes from postmortem studies. With increased iron levels are increased in the SNpc in PD, and the reduced levels of GSH where
excessive oxidative DAergic cell burden occurs which disrupts mitochondrial complex I function\textsuperscript{91,89}.

**Iron:**

Iron is richest in the basal ganglia with the highest levels being in the SNc, pallidus and putamen\textsuperscript{92,86}. In IPD, the shift of iron Fe\textsuperscript{2+}/Fe\textsuperscript{3+} ratio is almost 2:1 to 1:2\textsuperscript{93}, this is consistent with the increased Fe\textsuperscript{2+} -catalysed conversion of H\textsubscript{2}O\textsubscript{2} to the highly reactive OH\textsuperscript{-}. Known as the Fenton reaction\textsuperscript{86}:

\[
\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^- + \text{Fe}^{3+}.
\]

The reduction of Fe\textsuperscript{3+} by the superoxide radical increases the reaction rate which drives the production of OH\textsuperscript{-}. Iron levels are increased in IPD by about 35% specifically in the SNc \textsuperscript{94,89}. Similar increases have also been noted in other neurodegenerative diseases\textsuperscript{95,96}. Iron participates in the free-radical generating reaction only in the free ferrous form\textsuperscript{97}. Ferric iron (Fe\textsuperscript{3+}) in the SNc is normally bound either by ferritin (about 90%) or neuromelanin (NM) and, is associated with both LB and NM in IPD\textsuperscript{98}.

Infusion of iron into rat brains produces a model of PD characterized by a concentration-dependent loss of striatal DA, and the degeneration of SNc neurons with the behavioral changes\textsuperscript{70,99}.

Exposures to welding fumes have been shown to alter trace metal levels such as, manganese, iron, zinc and copper in the body. Serum levels of iron in the welders were 1.9 fold higher than those of controls and the level of erythrocytic superoxide dismutase activity was 2.4% less thus, occupational exposure to these toxic fumes disturbs the homeostasis of trace elements in the systemic circulation which induces OS\textsuperscript{100}. 
Neuromelanin (NM):

It’s been hypothesized that NM may act as an endogenous storage molecule for iron and possibly influence the production of free radicals. Iron-binding studies have demonstrated that both NM and synthetically-produced DA-melanin contained equivalent numbers of high and low-affinity binding sites for iron but, that the affinity of NM for iron was higher than that of synthetic melanin\textsuperscript{101}. The iron-binding capacity of NM is 10-fold greater than that of the synthetic melanin. This is consistent with the hypothesis. The function or effect of NM on neuronal function is unclear; it is generally regarded as a waste product of DA auto-oxidation, and accumulates with age in nigral neurons\textsuperscript{102}, but is dramatically decreased in PD patients\textsuperscript{103}. NM has been described as a ‘double-edged sword’ with respect to its sequestering of redox-active metal ions such as iron\textsuperscript{103}, induced by free radical reactions\textsuperscript{104} and inhibiting lipid peroxidation as an oxidant\textsuperscript{105}. NM may also promote ROS-generating and act as a depot for cellular toxins like MPP\textsuperscript{+106}.

Glutathione (GSH):

A defect in one or more of the naturally occurring antioxidants defenses could lead to the neurodegeneration in PD\textsuperscript{107}, although no basic defects have been detected in levels of ascorbic acid, \( \alpha \)-tocopherol, catalase, or glutathione peroxidase\textsuperscript{83}. The cellular radical detoxification system (the most important being superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity unfortunately declines with the ageing process. The activity of SOD is increased in discrete regions of the brain according to different neurodegenerative diseases, in IPD it is characteristically increased in SNc\textsuperscript{108}. This increase may be a neuroprotective response to the elevated levels of OS however; an increase in \( \text{H}_2\text{O}_2 \) production might be a problem in
the absence of corresponding catalase and GPX activity. In genetically modified rodents, high SOD expression protected the rat nigral cells against MPTP and 6-OHDA\textsuperscript{109}.

A Reduction in GSH impairs H\textsubscript{2}O\textsubscript{2} clearance thus, promoting OH\textsuperscript{•} formation, particularly in the presence of high levels of iron. The cause of GSH reduction in PD is still unknown and no defects have been detected in the enzymes associated with GSH synthesis. In the normal human brain, nigral GSH levels are low in comparison to with other brain regions\textsuperscript{93}.

**Mitochondrial dysfunction:**

There is a significant body of evidence which supports the hypothesis that the progressive reduction in mitochondrial respiration (a feature of the ageing brain) is involved in a number of neurodegenerative diseases including IPD\textsuperscript{110, 111}. A selective 38-49 % decrease in complex 1 activity of the mitochondrial respiratory chain has been found in the SNc of the PD patients\textsuperscript{111, 112}. The cause of the reduced complex I activity in IPD remains unknown.

Factors that contribute to the progression of PD include brain defects in mitochondrial respiration and the generation of H\textsubscript{2}O\textsubscript{2} by MAO. One study revealed that these two factors were linked\textsuperscript{113}.

**Nitric oxide (NO):**

Increased NO release from glial, microglia or macrophages could be responsible for the elevation of local OS\textsuperscript{114}. Although NO is an effective free radical scavenger\textsuperscript{115}. 

It can also react with the superoxide radical to form the peroxinitrite anion (ONOO−). The oxidative radical ONOO decomposes to form OH− + NO2, which are potent initiators of lipid peroxidation\textsuperscript{116}. NO also directly inhibits mitochondrial respiration at the level of complex IV and I, by liberating ferritin-bound iron thus, promoting lipid peroxidation\textsuperscript{117}, with mitochondrial respiration chain being damaged by sustained exposure to NO and that GSH is an important defense. This has implications for PD where GSH levels are decreased.

**Inflammation:**

Inflammation plays a role in the pathogenesis of PD. Autopsy reports have shown a glial response found in both ST and nigra of PD patients and MPTP-mice which may likely exert deleterious effects on the remaining DAergic neurons\textsuperscript{118}. This view has led many investigators to aggressively examine the potential role of neuroinflammation in the pathogenesis of PD. The SN DAergic neurons are vulnerable to a variety of insults due to their reduced antioxidant capacity with a high number of microglia cells, compared to other areas of the brain\textsuperscript{72}. When microglia becomes activated they release proinflammatory factors and such cytokines as interleukin-1 (IL-1), IL-2, IL-6, and tumor necrosis factor alpha (TNFα), which can be cytotoxic\textsuperscript{119}. Not only is there an indication of cytokine elevation but a decrease in neurotrophins such as BDNF and nerve growth factor (NGF), in the nigrostraital DA regions and ventricular and lumber cerebral spinal fluid of PD patient\textsuperscript{120}. Activated microglia and increased levels of inflammatory mediators have been detected in the ST of PD patients, *in vivo*\textsuperscript{120}, and *in vitro* studies\textsuperscript{121}. Which all point to an inflammatory component of DAergic cell loss. Acute toxin exposure in these models or decades after drug use in
humans have shown that the presence of activated microglia still persists, suggesting a brief insult can induce ongoing inflammatory response\textsuperscript{121}.

**2.1.7. Cell Death in PD:**

**Apoptosis:**

A recent study by Tatton\textsuperscript{122} shows that there is an increased occurrence of DNA fragmentation and chromatin condensation in the melanised cells of SN of PD patients compared to controls. There was also an increase in caspase-3 and Bax (a proapoptotic member of Bcl-2 protein family) immunoreactivity and nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase (GADPH) in PD nigral DAergic neurons. This demonstrates the occurrence of ongoing apoptotic processes and an involvement of mitochondrial processes.

**2.1.8. Current Treatments and Potential Therapies for PD:**

**Symptomatic treatment:**

There is no cure for PD, since currently available therapies can neither arrest or reverse the progression of the disease nor provide neuroprotection. However, the motor symptoms can be initially managed with several different drugs. The drugs used to treat PD either boost the levels of DA in the brain or mimic the effects of DA. The use of L-DOPA which is still considered the gold standard aims to relieve PD motor symptoms by replacing the deficient neurotransmitter, DA. However, the therapeutic efficacy of L-DOPA tends to fade with time and the development of motor and / or psychiatric side effects. An issue that is currently debated is the hypothesized toxicity of L-DOPA. Experimental data suggest that prolonged use of the drug may contribute to the degenerative process of PD.
various *in vivo* and *in vitro* studies have shown that L-DOPA can be toxic, as a result of its pro-oxidant properties\textsuperscript{123}. Whether L-DOPA is toxic per se or because it converts to DA whose metabolism is associated with the formation of ROS, as well is unclear \textsuperscript{124}.

These considerations have led to the introduction of DA agonist in PD therapy. This class of drugs, that includes compounds such as Pergolide, Bromocriptine, Lisuride or the more recent ropinirole and pramipexole, acts directly on the DA receptors mostly of the D\textsubscript{2} type mimicking the effect of DA. DA agonists reduce the occurrence of motor complications, in both experimental and clinical studies and are used as an adjunct to L-DOPA in patients with dyskinetic movements and fluctuations in the motor response to the drug\textsuperscript{125, 126} by restoring the normal efficiency of the DAergic pathways. Evidence suggests that DA agonist may have also neuroprotective properties. Such properties would be related to ascertained efficacy of DA agonists in antagonizing OS, at various levels\textsuperscript{127}. Apart from levodopa, drugs that are currently prescribed for the management of PD include DA receptor agonists, Selegiline, Amantadine, Catechol-O-methyl transferase (COMT) inhibitors and anticholinergics. Most current drugs are still in development and/or clinical phase and continuous research is employed to produce the most effective treatments which will have all desired effects such as neuroprotection, less severe side effects, optimal delivery of drugs to site of problems, relieve/ reduce motor symptoms and delay the neuronal degeneration.

**Cell transplantation:**

Transplantation of DA-producing neurons to replace those degenerated during the pathogenesis of PD is a promising approach to
treatment. Thus far this is the only advancement that has shown the capacity to allow patient to achieve full restoration of their functional capacity. The grafts have shown minimal immunological rejection in recipients and in the most successful trails have even allowed patients to withdraw from levodopa therapy\textsuperscript{128}. However; the majority of studies have derived allogenic ventral mesencephalic (VM) tissue from human foetuses\textsuperscript{129,130}. This presents, apart from the ethical issues, the implication that sources of such tissues will be limited and cannot provide a consistent and reproducible response. Furthermore substantial quantities are required to be adequately efficacious. Stem cells as alternative neuronal sources are receiving a great deal of focus as they can provide an unlimited source of tissue that can be employed to generate mature DAergic neurons to innervate the affected ST\textsuperscript{131}. The most promising is the bone marrow stem cells and ordinary epithelial skin cells\textsuperscript{132} may possess the potential to be sources of inducible stem cells. Although studies have shown promising results in preclinical trails, generated neurons have survived poorly after transplantation in animal subjects\textsuperscript{130}. One of the most difficult hurdles to overcome is the poor rate of graft cell survival as 90% of the transplanted cells fail to survive following intracerebral grafts\textsuperscript{133}. Furthermore, few studies have been able to investigate the long term implications of transplantation; thus the safety of such treatment at this stage remains questionable.

**Gene therapy:**

Transplanted neurons in PD patients are a potential target for the development of gene therapy procedures. A variety of different viral and non-viral methods for achieving gene delivery have been described\textsuperscript{134}. Among the numerous potential genes that have been evaluated for therapeutic efficacy
for PD, those encoding tyrosine hydroxylase (TH), guanosine triphosphate cyclohydrolase I and aromatic I-amino acid decorboxylase all allow for an increase in the production of DA\textsuperscript{135}.

Thus the development of a stem cell that has the capacity to differentiate into DAergic neurons as well as produce such factors is much needed\textsuperscript{131}. Genes encoding for the vesicular monoamine transporter-2 and glutamic acid decorboxylase have also demonstrated some benefit. Kang\textsuperscript{136} investigated potential genes that would be optimal for such therapy and found that the enzyme TH, which has been used in earlier studies, functions only when the essential cofactor, tetrahydrobiopterin is present. Thus, new developments into the delivery of genetically modified cells that can convert levodopa to DA and store it for gradual release is required. Alternatively, it has been proposed that nanomaterials like nanotubes may be employed therapeutically as ligand carriers or vectors for drug, DNA or gene delivery for PD\textsuperscript{137}.

**Surgical methods:**

Prior to the commercial availability of L-DOPA, treatment for PD emphasized surgical intervention\textsuperscript{138}. Such intervention such as thalamotomy focused on the reduction of tremor, but failed to address the more debilitating symptom, bradykinesia\textsuperscript{138}. However, pharmacological therapy for PD often becomes in adequate over long term use and during significantly progressed stages of the disease. The patient’s disability increases despite maximal drug management and many patients develop motor fluctuations and dyskinesias. Surgical interventions for PD have been shown to be beneficial for refractory symptoms. However their role is limited to being the
means of last resort’ due to the high risk of potential complication and limited long term efficacy. Recent advances in this field have provided the greater range of surgical options. Thalamotomy, pallidotomy are destructive lesions thought to improve motor deficient. Deep brain stimulation (DBS) However offers subthalamic nucleus stimulation improves levodopa-induced ‘off’ period function, decreases ‘off’ time, and reduces dyskinesia\textsuperscript{139}. Surgery is considered for people with intolerable adverse side effects from medication, and those patients who have significant cognitive capacity\textsuperscript{140}.

Possible adverse side effects of surgery include brain haemorrhage, infarction, seizures, and even death\textsuperscript{141, 142}. Furthermore, successful surgical DBS procedure can still lead to side effects that include worsening dyskinesia, paraesthesias, speech, and gait disturbances\textsuperscript{143}. These systems promise to allow greater safety and precision for the delivery of impulses in the SN, and therefore reduce side effects the aforementioned problems with regard to surgery and equipment malfunctions, Studies are still in preliminary phases of development for eliminating repeated surgeries; however animal studies have yielded exceedingly promising results.

2.2. Description of *Nardostachys jatamansi* DC\textsuperscript{144, 145,146,147}:

*Nardostachys jatamansi* is well known in India from times immemorial. In ancient Indian scriptures like Rigveda and Athavaveda its uses are mentioned in charak & sushuruta samhitas ca 1000–800 BC and by the unani physicians in their ancient books.

*Nardostachys jatamansi* was not only known in India but also in Egypt, Greece, Rome and Arabian countries as an important commodity from India from times immemorial. It was reputed for its various uses such
as costly incense, a nerve tonic and use in hysteria, epileptic fits, palpitation of heart, etc., in ancient history right from the period of atharvaveda and its export from India ca 2500 B.C to Mesopotamia, where it is recorded in wedge shaped cune form script. Its association with Alexander the great. Its inclusion in the bible plants and its use as a royal perfume during reign of Akbar the great.

*Nardostachys* is a small Herbaceous Himalayan Jenus, represented by two broad range endemic species, *Nardostachys grandiflora* DC and *Nardostachys jatamansi* DC, in India *Nardostachys grandiflora* has been reported to be scarcely occurring in certain localities. (Above 4000m) of kumaon and Sikkim Himalaya where as *Nardostachys jatamansi* is relatively common in Garhwal, kumaon and Sikkim Himalaya between 3000 and 5000m.

The underground part of *Nardostachys jatamansi* is used as a substitute for valerian and the extracts find use in over 26 ayurvedic preparations. The root is also used for treatment of heart disease, high blood pressure and insomnia. The root and rhizome contains active components with carminative, sedative, antispasmodic and tranquilizing properties.

**Family:** Valerianaceae.

**Vernacular names:**

Sanskrit & Bengali - Jatamansi

Hindi - Jatamansi, Bal - chir

English - Indian Spike Nard, musk – root

Marathi - Jatamavshi’
Gujarathi - Jata-masi, Kalicchad
Telugu, Kannada & Malayalam - Jatamamshi
Tamil - Jatamashi
Kashmir - Bhutijatt, Kukilipot
Nepal - Naswaa, Sinhalese, Jatamansi
Garhwal - Masi
Bhutan - Pampe, Jatamansi
Chinese - Kan Sung
German - Indische Narde
Arabian - Sunbulatteb
Persian - Sambul -e- Hindi
Tibetan - Span - pos
Malaysian - Jata – manchi
French - Nard Indian

**Distribution and occurrence:**

In India, in kumaon Himalayas (Uttarkashi, Chamoli, Tehri, Bageshwar & Piphoragarh districts), in Eastern Himalayas (Sikkim & NEFA). In other parts of world it’s found in central Himalaya (Nepal), Eastern Himalaya (Bhutan), and East North Himalayas (Tibet & China).

**Parts used:** Rhizomes & roots.
Fig: 2.1 Exomorphic features of the *Nardostachys jatamansi* (DC).

**Macroscopic characters:**

The plant bears a stem 10 to 60cm long which is more or less pubescent upward and often glabrate below. Rhizomes stout, long, woody, covered with fibers from the petioles of withered leaves. Leaves usually in pairs, 2.5 to 7.5cm long, sessile, oblong, or subovate. Flower heads usually 1, 3 or 5 with pubescent bracts. Corolla 5-limbed, somewhat hairy with in, corolla tube 6mm long. Filaments hairy below. Fruit covered by ovate, acute, often dentate calyx teeth, with ascending white hairs. The fibers are produced by an accumulation of the skeletons of the leaves and are matted together forming a kind of network. Internal color is reddish brown. Odor is highly aromatic.

**Microscopic characters:**

A transverse section of the rhizome shows a brown bark separated by a cambium from the porous wood. The periderm consists of two to eight layers of cork cells. These layers occur in the outer cortex. Phellogen is not distinguishable. The secondary cortex is characterized by the presence of prominent oleoresin cells containing oil and resinous matter. The phloem consists of a number of cells having a diameter of about 10μm. Cambium
ring is distinct. The wood is characterized by the presence of numerous vessels scattered almost uniformly. Medullary rays are not prominent. The rest of the wood is composed of tracheids and a few fibers. Vessels are mostly scalariform thickening and few with spiral thickening. Vessels with irregular forms also present. Average dimensions of vessels are 40 to 50μm. The distinguishing characteristics under microscope as the presence of interxylary and medullary cork, schizogenous cavities in the young rhizome in the inner cortex, and parenchyma of phloem and xylem, but without any epithelial cells and oil globules in cork cells.

**Chemical constituents:**

The sesquiterpene valeranone (= yatamanson) was isolated from the subterranean parts of NJ which is having sedative, tranquilizing, antihypertensive properties, prolongation of barbiturate hypnosis, the impairment of rota rod performance, an anticonvulsive activity on electric shock and potentiation of the body temperature lowering activity of reserpine and antiulcer activity\textsuperscript{148}.

A new terpenoid ester, nadostatchysin (1), isolated from the rhizomes of NJ were established as the 7', 8'-dihydroxy-4'-methylene hexa hydro cyclopenta[c]pyran-1'-one-8'-methylester of 7, 9-guaiadicon-14-oic acid\textsuperscript{149}.

The rhizome volatiles contained 72 components of which 41 constituents comprising 70% of the oil were identified. The oil composed of nine monoterpenes (1.7%), 25 sesquiterpenes (43.9%) and 7 non terpenic components (24.4%). The predominant sesquiterpenes were nardol (10.1%) alpha-selinene (9.2%), beta-caryophyllene (3.3%), cubedol (2.9%), alpha-gurjunene (2.5%), gamma-gurjunene (02.3% and alpha-humulene (2.3%)\textsuperscript{150}. 

Reported pharmacological activities on *Nardostachys jatamansi*:

**Antidiabetic activity**\(^{151}\):

The extract of *Nardostachys jatamansi* shows the protective effect against cytokine-induced beta-cell damage and streptozotocin-induced diabetes.

**Acute pancreatitis**\(^{152}\):

*Nardostachys jatamansi* protects against cerulein-induced acute pancreatitis.

**Effect on CFS**\(^{153}\):

The antioxidant property of *Nardostachys jatamansi* causes the alleviation of the symptoms of the chronic fatigue syndrome.

**Stress modulating effect**\(^{154}\):

The anti-stress effect of hydroethanolic extract (70%) of NJ was evaluated in reference to its antioxidant property.

**Antifungal activity**\(^{155}\):

Six essential oils isolated from NJ were having fungistatic (or) fungicidal activity.

**Antioxidant and lipid peroxidation activity**\(^{156}\):

The extracts of NJ at a dose of 500 mg/kg for seven days showed a significant changes in the antioxidant enzymes (superoxide dismutase,
catalase, glutathione peroxidase and glutathione-S-transferase) and lipid peroxidation levels in doxorubicin (15 mg/kg, i.p) induced cardiac damage in rats.

**Anticonvulsant and neurotoxicity**\(^{157}\):

Ethanolic extract of NJ root extract against maximal electroshock seizure (MES) model decreased the extension/flexion ratio, also showed the minimal neurotoxicity against rota rod test. Pretreatment of rats with phenytoin at doses of 12.5, 25, 50 and 75 mg/kg in combination with 50 mg/kg of NJ increased in the protective index (PI) of phenytoin from 363 to 13.18

**Smooth muscle relaxant activity**\(^{158}\):

Extracts of NJ showed significant effect on constrictor response of histamine, acetylcholine and serotonin on smooth muscles.

**Antiarrhythmic activity**\(^{159}\):

The extracts of NJ showed significant antiarrhythmic activity.

**Learning and memory in mice**\(^{160}\):

The ethanolic extract of NJ at a dose of 200 mg/kg for 8 successive days improved learning and memory and also reversed the amnesia induced by diazepam (1 mg/kg, i.p.) and scopolamine (0.4 mg/kg, i.p.)

**Anti asthmatic activity**\(^{161}\):

NJ fumes and aerosols significantly ameliorated the bronchial asthma in guinea pigs induced by histamine.

**Hypolipidaemic activity**\(^{162}\):
50% ethanolic extract of curcumalonga (rhizome) and NJ (whole plant) feeding elevated HDL cholesterol/ total cholesterol ratio. The extracts also caused a significant reduction in the ratio of total cholesterol/phospholipids.

**Hypotensive effect**: The essential oil isolated from NJ caused the prolongation of hypotensive effect.

**Lung perfusion and tidal air changes**: *Nardostachys jatamansi* and Rhus succedanea showed protective effect against histamine and serotonin response on lung perfusion and tidal air changes.

**Effect of Biogenic amines and inhibitory amino acids**: Alcoholic extract of the roots of NJ at a dose 250 mg/kg for 15 days treatment resulted in a significant increase in the levels of NE, DA, 5-HT, 5HIAA and GABA in rat brain.

**Antiepileptic activity**: Antiepileptic activity of phenytoin was enhanced with the administration of a polyherbal formulation Shankhapushpi containing (Convolvules pluricaulis, Centella asiatica, Nepata hindostana, NJ, Nepeta elliptica and Onosma bracteatum).

**Sedative and tranquilizing properties**: Medya drug like ashwagandha, brahmi, jatamansi etc., (for the treatment of psychological and psychomatic disorders) act as the
sedative and tranquilizer and also they keep the mind calm and cool, reduce anxiety and apprehension.

**Effect on rat cerebral ischemia**\(^{168}\):

The extract of NJ at a dose of 250 mg/kg for 15 days treatment in middle cerebral artery (MCA) occlusion model of acute cerebral ischemia in rats significantly ameliorated the levels of glutathione, thiol groups and the levels of TBARS. The effect is probably by virtue of its antioxidant property.

**Antilipid peroxidation activity**\(^{169}\):

Ethanolic and hexane extracts of *Nardostachys Jatatamansi* showed antiperoxidative property in iron induced lipid peroxidation in 5% rat liver homogenate. Hexane extract was more potent.

**Effect on insomnia**\(^{170}\):

A polyherbal preparation containing NJ at a dose of 2 tablets per day for 21 days at bed time, significantly increased the total sleep time. Total sleep time increased from 204 31.00 min to 262 38.6 min.

**Effect on oxidative stress, toxicity, ear edema**\(^{171}\):

Acetone extract of NJ at the doses of 2.5 and 5 mg/kg prior to the application of benzoyl peroxide (20 mg/animal per 0.2ml acetone) resulted in significant inhibition of benzoyl peroxide induced cutaneous oxidative stress, toxicity and ear edema.

**Sleep onset insomnia**\(^{172}\):
A traditional ayurvedic supplement containing NJ is reported to have significant decrease in sleep latency of 16.72 mints (S.D = 44.8) compared to placebo (p=0.003).

**Medicinal properties and uses**:

Roots of *Nardostachys jatamansi* are used as an aromatic adjunct in the preparation of medicinal oil and in perfumery. It is a good substitute for the official valerian. Its infusion is employed in the treatment of spasmodic hysterical infections, mainly palpitation of the heart, nervous headache, and flatulence in doses of 28 to 56 ml three times daily. It is used in diseases of the digestive and respiratory organs and in jaundice. It is said to be useful also in leprosy. Mixed with sesamum oil, it is rubbed on the head as a nerve sedative. It also promotes growth and blackness of hair. The drug is prescribed as an antidote in scorpion sting.

**2.3. Description of Smilax zeylanica Linn:**

*Smilax Zeylanica* Linn. is an evergreen woody climber endemic to Western Ghats of Southern India. The genus Smilax Linn. consists of more than 300 species, distributed all over the world, out of which 24 are found in India, in south India, 4 species, viz. *smilax aspera* Linn., *Smilax perfoliata* Lour., *Smilax wightii* A. DC. and *Smilax zeylanica* Linn. occur. *Smilax zeylanica* is used in the treatment of abscesses, skin disorders, sores, swellings and venereal diseases. Different species of the genus including *Smilax zeylanica* are also used as substitutes for Sarsaparilla in many parts of the world. The phytoconstituents reported in the leaves and roots of *Smilax zeylanica* are steroidal saponin glycosides like diosgenin, smilagenin and sarsapogenin.

**Family:** Smilacaceae.
Fig: 2.2 Exomorphic features of the *Smilax zeylanica* Linn.

The vernacular names of *Smilax zeylanica* are:

<table>
<thead>
<tr>
<th>Language</th>
<th>Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hindi</td>
<td>Chobchini, Jangliaushbah, Ramdatun;</td>
</tr>
<tr>
<td>Kannada</td>
<td>Kaadu hambu thaavare;</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Chopachinee, Vanamadhusnuhi;</td>
</tr>
<tr>
<td>Telugu</td>
<td>Kummeritheega, Kondadantena, Kondagarbhat hige, Konda, Sithapa, Kondagarbhathige, Konda, Sithapa, Gurivatheega, Kondathaamara, Kummarabaddu, Kushtaputamara;</td>
</tr>
<tr>
<td>Tamil</td>
<td>Ayadi, Tirunamappalai, Periyakanni, Karuvilanchikudam, Kaattukkodi, Malaitthamarai;</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Cherunchakayagavalli, Kalthamara, Karivilanti;</td>
</tr>
<tr>
<td>Marathi</td>
<td>Gholbel, Gutwel, Guti;</td>
</tr>
<tr>
<td>Bengali</td>
<td>Kumarik.</td>
</tr>
</tbody>
</table>

*Smilax zeylanica* Linn. is a dioecious climbing shrub; stem woody,
sometimes armed with prickles; branchlets angular. Leaves 20 × 16 cm, variable in shape (ovate, elliptic or oblanceolate), acuminate at apex, rounded at base; petiole up to 3 cm long; leaf sheath 8 mm long, narrow. Flowers greenish-white, in umbels: umbels 1 to 3. Perianth-lobes 6. Stamens 6, subequal. Pistillode absent. Ovary 3-locular; style short; stigmas 3; staminodes 3 (rarely 6), equalling stigmas. Berry globose.

**Distribution & occurrence:**

Distributed in temperate zones, tropics and subtropic world wide. In India it is endemic to the western ghats of south India. It is a slow growing riparian species distributed up to 1200 m altitude. In other parts of world it’s found in China, North America.

**Macroscopic characters:45**

Rhizomes 5 to 7 cm long and 5 cm thick, woody, brownish to blackish externally, yellowish internally, rough, give rise to several roots at different points, fracture fibrous, without characteristic odour and with slightly bitter taste.

Roots 10 to 20 cm long and 2 mm thick, adventitious, several, arising from different points of rhizome, run either vertically or horizontally, wiry, very hard, surface smooth, brownish to blackish externally, yellowish internally, without any characteristic odour and with slightly bitter taste, fracture fibrous. Fresh young roots are white.

**Microscopic characters:45:**

Transverse section of rhizome exhibits epidermis, a wide yellowish ground tissue, where vascular bundles are found scattered. Epidermis is
single layered, made up of compact rectangular cells. Next to epidermis lies a large ground tissue which can be differentiated in to two regions. Outer ground tissue is parenchymatous, many layered, consists of oval, thin walled cells, measure 24-40-45µ long; some cells are tanniferous, some others contain raphide bundles consisting of acicular crystals while in a few other cells granular cell contents are found; each raphide measure 6.6-6.7-6.9µ long; lysigenous cavities are found scattered in outer ground tissue. Inner ground tissue consists of an outer one layered sclerenchyma, which consists of large, thick walled cells having large lumen, measuring 17-27-45 × 15-26-47µ; this is followed by a few layers of smaller compactly arranged sclerenchyma cells, measuring 43-48-62 × 26-35-54µ while the remaining part of ground tissue is parenchymatous; some cells of this region contain dark content which are tanniferous and some others yellow cell content. Vascular bundles are found scattered; they are conjoint, collateral, encircled by a sclerenchymatous bundle sheath; a few lysigenous cavities are conspicuous.

Transverse section of root is circular in outline. Young fresh root exhibits a single layered epidermis made up of compactly arranged rectangular cells measuring 12.5-19-26 × 12-18-26µ. This is followed by many layered cortex and stellar region with central pith. Cortex is broad, multilayered, consisting of parenchymatous cells measuring 21-54-75 × 26-49-71µ having intercellular spaces. In mature roots the epidermis and cortex peels off very early. Transverse section of mature root shows endodermis which is blackish, one layered cells measure 23-31-35 × 16-20-23µ. Pericycle lies next to the endodermis which is sclerenchymatous with cells having large lumen, measuring 10-30-43 × 6-15-22µ. Xylem and phloem are
radically arranged, found embedded in the sclerenchyma; xylem is exarch, measure 7-13-8 × 7-12-16μ. Vessels measure 30-41-49 × 20-27-34μ; some vessels contain darkly stained cell contents. Phloem cells measure 93-120-167 × 58-65-80μ, consists of phloem parenchyma, sieve tubes and companion cells. Pith is large, consists of parenchymatous cells with intercellular spaces, measuring 22-49-72 × 26-41-58μ. Raphide bundles of acicular crystals occur in some larger pith cells; each raphide measuring 43-57-82 × 3.2-3.7μ; some pith cells are pitted. Starch grains are simple, found in some pith cells and phloem parenchyma, measure 3.5-7-9μ (diameter).

**Chemical constituents:**

The phytoconstituents reported in the leaves and roots of *Smilax zeylanica* are steroidal saponin glycosides like diosgenin, smilagenin and sarsapogenin177.

**Medicinal uses178:**

The roots of *Smilax zeylanica* are used in the treatment of venereal and skin diseases, sores, swellings, abscesses, diseases of nervous system, epilepsy, psychosis, urinary disorders, polyurea, wasting disease, hemipleagia, parkinsonism, congenital diseases, leprosy, rejuvenator.

**Reported pharmacological activities on Smilax zeylanica:**

**Antidiabetic activity179:**

The Methanol extract of *Smilax zeylanica* has shown antidiabetic activity in streptozotocin induced diabetic rats by oral administration of
extract 400 mg/kg body weight for 15 days.

**Antioxidant activity**

The antioxidant property of *Smilax zeylanica* Linn. shows the hepatoprotective effect in CCl4 induced hepatotoxic in rats by oral administration of Methanol extract of *Smilax zeylanica* roots and rhizomes at a dose of 200, 400 and 600 mg/kg/day, p.o. for 7 days.