6. DISCUSSION OF THE RESULTS

Ethnopharmacology and drug discovery using natural products remain important issues in the current target-rich and lead-poor scenario\textsuperscript{24}. Many modern drugs have their origin in ethnopharmacology. Globally, there is a positive trend in favor of traditional and integrative health sciences in both research and practice. There are common approaches to drug discovery including the use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics. However, the innovative approaches involve ethnopharmacology, reverse pharmacology, holistic, system biology and personalized medicine. There are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches\textsuperscript{205}. The ethnopharmacology knowledge and experimental base allows drug research from clinics to laboratories - a true reverse pharmacology approach. In this process, ‘safety’ remains the most important starting point and the efficacy becomes a matter of validation. A golden triangle consisting of traditional knowledge, modern medicine and modern science with systems orientation will converge to form an innovative discovery engine for newer, safer, affordable and effective therapies\textsuperscript{206}.

An analysis of the origin of the drugs developed between 1981 to 2002 showed products of natural product-derived drugs comprised 28\% of all new chemical entities launched into market\textsuperscript{207}. In addition, 24\% of these new chemical entities were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural products\textsuperscript{208}. This combined percentage (52\% of all new chemical entities) suggests that natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development.
Pharmacognostic studies are useful in developing histological standards of raw materials and its characteristics, by what means, we can differentiate, the genuine and authentic samples from the adulterated samples. This would help in developing herbal monograph and standardize the raw materials used in the formulation.

Preliminary phytochemical studies confirmed the presence of alkaloids, carbohydrates, proteins, steroids, sterols, tannins, flavonoids, gums and mucilage, glycosides, saponins and terpenes in EENJ, alkaloids, carbohydrates, steroids, sterols, tannins, flavonoids, gums and mucilage, glycosides and terpenes in HAENJ and carbohydrates, steroids, sterols, flavonoids, gums & mucilage and terpenes, in AENJ.

The EESZ demonstrated the presence of carbohydrates, proteins, steroids, phenols, flavonoids, gums and mucilage, and saponins.

The TLC of EENJ, HAENJ, AENJ and EESZ has shown the various spots at wavelengths 254 nm, 366 nm in solvent systems of petroleum ether: acetone (3:1), toluene: ethyl Acetate: methanol (7:2:1) and toluene: ethyl acetate (7:3). Which represent the presence of various types of constituents in the different extracts of NJ and SZ.

The HPTLC finger print of EENJ at wavelength 254 nm showed the presence of 8 spots in solvent system of petroleum ether: acetone (3:1) and 7 spots in solvent system of toluene: ethyl acetate: methanol (7:2:1). At wavelength 366 nm in solvent system of petroleum ether: acetone (3:1) 8 spots and 7 spots in solvent system of toluene: ethyl acetate: methanol (7:2:1).
The HPTLC finger print of HAENJ at wavelength 254 nm showed the presence of 8 spots in solvent system of petroleum ether: acetone (3:1) and 7 spots in solvent system of toluene: ethyl acetate: methanol (7:2:1). At wavelength 366nm in solvent system of petroleum ether: acetone (3:1) 6 spots and 6 spots in solvent system of toluene: ethyl acetate: methanol (7:2:1).

The HPTLC finger print of AENJ at wavelength 254 nm showed the presence of 6 spots in solvent system of petroleum ether: acetone (3:1) and 6 spots in solvent system of toluene: ethyl acetate: methanol (7:2:1). At wavelength 366nm in solvent system of petroleum ether: acetone (3:1) one spot and 2 spots in solvent system toluene: ethyl acetate: methanol (7:2:1).

The HPTLC finger print of Smilax zeylanica at wavelength 366 nm in solvent system toluene: ethyl acetate (7:3) showed the presence of 9 spots in ethanol extract, 7 spots in hydro alcohol extract and 3 spots in aqueous extract.

EENJ, HAENJ, AENJ and EESZ were subjected to in-vitro antioxidant studies using nitric oxide and hydrogen peroxide free radical scavenging models. EENJ and EESZ were showed moderate activity with IC_{50} value 734.37 µg/ml, where as hydro alcoholic and aqueous extracts did not show the activity at the tested range for nitric oxide scavenging. Among the test drugs tested, all the extracts showed potent antioxidant activity with IC_{50} values ranging from 160 to 200 µg/ml for scavenging of H_{2}O_{2}.

Acute toxicity of EENJ, HAENJ, AENJ and EESZ was tested in male Wister rats. Body weights before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, respiratory, circulatory,
autonomic, central nervous system, somatomotor activity, behaviour pattern were observed, sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were seen. The onset of toxicity and signs of toxicity were not seen even at a dose 5 grms/kg orally. It has been found that these extracts were safe to use to animals even at a dose 5 gm/kg orally.

Repeated oral toxicity studies of EENJ, HAENJ, AENJ and EESZ tested in rats at a dose of 1000 mg/kg body weight p.o for 28 days. No considerable changes in haematological parameters and histopathology of organs was observed. Hence, the 100mg/kg, 250 mg/kg and 500 mg/kg doses selected for pharmacological studies.

The present study demonstrates the neuropharmacological effects of EENJ, HAENJ, AENJ, and EESZ in haloperidol model of Parkinson’s disease in rats.

Haloperidol induced catalepsy in rats is used to screen the drugs for their antiparkinsonism effects. In the present study the EENJ, HAENJ, AENJ and EESZ were screened for its effects in haloperidol induced catalepsy in rats.

Dopamine depletion is considered as a cardinal feature in causing of PD in humans or in animal models of the disease. The enhancement of dopamine content by Nardostachys jatamansi treatment might have restored the alteration in locomotor activity, exploratory behaviour\textsuperscript{168}.

Haloperidol inhibited the locomotor activity at the dose of 1 mg/kg/i.p. The inhibition of locomotor activity induced by haloperidol was decreased by the treatments of L-dopa+carbidopa, EENJ, HAENJ, AENJ and EESZ.
The EENJ, HAENJ, AENJ, and EESZ at doses of 100, 250 and 500 mg/kg showed the results similar to L-dopa +carbidopa (100 + 25 mg/kg) in ameliorating the haloperidol inhibited locomotor activity. The EENJ, HAENJ, AENJ, and EESZ at a dose of 500mg/kg exhibited significantly high protection against haloperidol decreased locomotor activity in rats. Among all the extracts of Nardostachys jatamansi, ethanol extract exhibited high potency and ethanol extract of Smilax zeylanica also exhibited high potency.

Alcoholic extract of NJ has been reported to have alternation of Parkinsonism symptoms induced by 6-hydroxydopamine in rats and alcoholic extract of roots of NJ restored the dopamine content of substantia nigra after its depletion with 6-OHDA, alcoholic extract of NJ treatment also restored the alterations in locomotor activity and muscle coordination in 6-OHDA rats.

Extensive evidences indicate that the part of PD symptomatology may be attributed to a dysfunctional NA system. Morphological changes in the locus corpuleus, an eminently nor adrenargic brain stem area has been reported in PD. Nardostachys jatamansi extract having NA enhancing property, may ameliorate freezing, disrythmia, all day life activity and mood in patients with PD.

Several studies have reported on exacerbation of neuroleptic-induced catalepsy by enhanced serotonergic neurotransmission in the CNS. Ondansetron by antagonizing the stimulatory effects of 5-HT₃ receptor activation on the release of dopamine decreases the levels of dopamine in the nigrostraited and mesolimbic pathways. The two main dopamine pathways in the brain are the mesolimbic pathway and the nigrostraited pathway.
Antipsychotic effect of haloperidol is believed to be achieved by inhibition of dopaminergic transmission in mesolimbic pathway. The haloperidol induced catalepsy in rats has been proposed to be a direct consequence of antagonism of dopamine D$_2$ receptors$^{213}$. Neuroleptics like haloperidol exerts multiple effects on dopaminergic signaling and produce DA related behavioral changes and catalepsy$^{214}$.

Cold restraint stress (RS) or forced immobilization stress is one of the most popular stressors in experimental medicine$^{215}$. The ascending serotonergic neurons from raphe nuclei are reported to innervate hypothalamic and limbic sites and have an overall role in the secretion of corticotropic hormone (ACTH) during stress$^{216}$. RS for 4 h enhances the levels of corticosterone in plasma$^{217}$. Ethanolic extract of NJ produced anti-depressant like effect in mice by inhibiting MAO-A and MAO-B, and through interaction with GABA ergic receptors$^{218}$.

FST induced immobility was decreased by the treatments of Fluoxetine, Venlafaxine, EENJ, HAENJ, AENJ and EESZ.

The EENJ, HAENJ, AENJ and EESZ at doses of 100, 250 and 500 mg/kg reversed the FST induced immobility, and exhibited similar pharmacological effects similar to Fluoxetine 20mg/kg, Venlafaxine 10mg/kg. The EENJ, HAENJ, AENJ and EESZ at dose of 500mg exhibited significantly high protection. Among all the extracts of *Nardostachys jatamansi* ethanol extract exhibited high potency and ethanol extract of *Smilax zeylanica* also exhibited high potency.

The haloperidal (a non selective D$_2$ dopamine antagonist) induced catalepsy is primarily due to blockage of dopamine receptors in the striatum. The degeneration of dopaminergic neurons leads to an increase in population of dopamine receptors$^{210}$.
Haloperidol induced catalepsy (rigidity, akinesthesia) at a dose of 1 mg/kg/i.p. The inhibition of haloperidol induced catalepsy was increased by the treatments of L-dopa+carbidopa, EENJ, HAENJ, AENJ and EESZ.

The EENJ, HAENJ, AENJ and EESZ at doses of 100, 250 and 500 mg/kg showed the results similar to L-dopa +carbidopa (100 + 25 mg/kg) in ameliorating the symptoms of haloperidol induced catalepsy. The EENJ, HAENJ, AENJ and EESZ at a dose of 500mg exhibited significantly high protection against haloperidol induced catalepsy in rats. Among all the extracts of *Nardostachys jatamansi* ethanol extract exhibited high potency and ethanol extract of *Smilax zeylanica* also exhibited high potency.

Previous studies have shown that dopamine receptors in the striatum are involved in neuroleptic-induced catalepsy\(^{219}\). It has been demonstrated that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localized postsynaptically on strial neurons\(^{219}\).

Haloperidol causes the changes in behavioural assessment like immobility, rigidity at a dose of 1 mg/kg/i.p. The inhibition of haloperidol induced immobility; rigidity was increased by the treatments of L-dopa+carbidopa, EENJ, HAENJ, AENJ and EESZ.

The EENJ, HAENJ, AENJ and EESZ at doses of 100, 250, and 500 mg/kg showed the results similar to L-dopa +carbidopa (100 + 25 mg/kg) in ameliorating the haloperidol inhibited mobility. The EENJ, HAENJ, AENJ and EESZ at a dose of 500mg exhibited significantly high protection against haloperidol induced immobility in rats. Among all the extracts of *Nardostachys jatamansi* ethanol extract exhibited high potency and ethanol extract of *Smilax zeylanica* also exhibited high potency.
The central nervous system is especially vulnerable to free radical damage because of the brain’s high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues. Evidence also indicates that ROS may stimulate extracellular release of excitatory amino acids. Glutamate is the major excitatory amino acid in the brain which acts through various types of ionotrophic receptors, the most significant being N-methyl D-aspartate (NMDA) receptors. There seems to be a bidirectional relationship between the ROS production and the release of excitatory amino acids. Free radicals generated in the brain are also reported to influence gene expression, subsequently effecting apoptosis and neuronal death. In the brain, an array of cellular defense systems exists to counterbalance the ROS. These include enzymatic and nonenzymatic antioxidants that lower the concentration of free radical species and repair oxidative cellular damage. The brain is known to synthesize molecules like glutathione and NADPH. Glutathione functions as a major antioxidant in tissue defense against free radicals in the brain. However, the concentration of glutathione is, relatively, in lesser quantities in the brain as compared to the other organs of the body. The natural antioxidant system present in brain can be in form of enzymes like catalase, peroxidase, superoxide dismutase or low molecular weight antioxidants (ascorbic and lipoic acids, carotenoids or indirectly acting chelating agents). Free radical scavengers or antioxidants function as biological bodyguards for essential molecules by either neutralizing reactive species before they mutilate a molecule or they repair damage that has been inflicted.

The present study demonstrates the antioxidant effects of EENJ, HAENJ, AENJ and EESZ in haloperidol-induced, cataleptic oxidative stress in rats.
The induction of free radicals in mammals by haloperidol is well established. It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue. The increase in SOD observed in the present study supports this concept. Superoxide formation is a major factor in oxygen toxicity and the superoxide dismutase enzyme constitutes an essential defense against it. Under normal conditions, decreased activity of antioxidant enzymes, such as SOD, glutathione peroxidase and catalase, in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects. An increase in these enzymes under normal conditions would represent increased antioxidant activity and a protective mechanism in neuronal tissue, thus, constituting the first line of defense against oxidative stress in our body. However, in the presence of a free radical-quenching agent, the induction of the antioxidant enzymes is minimized. So, any overall decrease in cataleptic scores and SOD activity in the drug treated groups indicates the ability of the drug extract to combat oxidative stress in brain tissue and reduce the severity of haloperidol-induced catalepsy. The altered balance of the antioxidant enzymes caused by the decrease in CAT, SOD, GSH activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage. The decreased activities of CAT and SOD may be a response to increased production of H$_2$O$_2$ and O$_2$ by the autooxidation, it has been suggested that these enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides. Treatment with EENJ, HAENJ, AENJ and EESZ increased the activity of these enzymes by quenching the free radicals. Previously *Nardostachys jatamansi* has been reported to be a well known antioxidant and the ethanol extract of *Nardostachys jatamansi* is reported to possess potent
antioxidant activity that scavenges free radicals generated after the induction of catalepsy\textsuperscript{227}. Significantly lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and nonenzymatic antioxidants in the brain suggest that the extract reduces oxidative stress.

The marked restoration of lipid peroxidation and enhancement of GSH content and antioxidant enzyme activities by pretreatment with alcoholic extract of \textit{Nardostachys jatamansi} has been reported\textsuperscript{168}.

Antioxidants play an important role in prevention and control of PD. Many authors have reported that Parkinsonism was partially protected by the application of antioxidants\textsuperscript{227}. Drugs that enhance the availability of dopamine or prevent its breakdown afford protection against PD in humans or in animal models.

Alcoholic extract of \textit{Nardostachys jatamansi} having dopamine enhancing property\textsuperscript{165}, and antioxidant potential property\textsuperscript{169, 168}, might have afforded protection in the haloperidol induced Parkinson's disease.

The haloperidol treated rats showed a significant increase in TBARS and there was also a significant reduction in SOD, CAT, and GSH in the brain tissue. Oral administration of the EENJ, HAENJ, AENJ and EESZ extracts at a dose of 100, 250, and 500 mg/kg along with haloperidol administration significantly restored the peroxides and antioxidant levels to near normal in the brains of the test animals.

The levels of dopamine were depleted significantly in haloperidol administered rats. Oral administration of the EENJ, HAENJ, AENJ and EESZ extracts at a dose of 100, 250 and 500 mg/kg along with haloperidol administration significantly restored the dopamine levels to near normal in the brains of the test animals.

For all the parameters studied, \textit{Nardostachys Jatamansi, Smilax zeylanica}
extracts administered at a dose of 500 mg/kg bodyweight showed significant effects. L-Dopa and carbidopa also showed a significant effect in all the parameters studied in rats.