Chapter 10

Summary and Conclusions
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*Nardostachys jatamansi* 70% ethanolic extract (NJE) was prepared and its metabolite profile was assessed using phytochemical screening for the quantification of polyphenols, flavonoids and its free radical and antioxidant activities were studied using, 2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), superoxide and metal chelating activities. The extract was found to be a potent scavenger of these ROS and it further inhibited DNA damage, protein oxidation, reactive oxygen species and protein carbonyl’s formation owing to its antioxidant potency.

Metabolite profiling of plant extracts yields critical information about their chemical composition. A comprehensive approach in analysing the metabolites present in NJE detailed the presence of an array of metabolites, all of which contribute synergistically to the varied biological effects attributed to the herb. Phytochemical screening using qualitative tests revealed the presence of an array of metabolites viz. tannins, sesquiterpenes, triterpenes, steroids, saponins, amino acids etc. RP-HPLC aided in identifying the major polyphenols in NJE and also revealed an enriched serotonin, melatonin content, GC-MS-HT-TOF analysis revealed the volatile components and fatty acid profile, LC-ESI-MS/MS showed an array of sesquiterpenes, monoterpenes, furfuran lignan, aristolane ketone, benzamides, mineral composition showed an array of minerals, amino acid composition showed an enriched Glx content, and HPTLC analysis for GABA showed an enriched GABA content in NJE. Data generated from this study was useful to answer important questions relating to the mode of action of the plant, in our study with respect to anxiety.

After analysing the metabolite profile, NJE was screened for its potential anxiolytic effects following an intraperitoneal injection and assessing the behaviour of mice in elevated plus maze test (EPM) and open field test (OFT). 250 mg/kg was found to be effective as it increased the time spent by mice on open arms of EPM and the number of line crossings in OFT with results comparable to standard anxiolyte diazepam (1 mg/kg). Hence, 250 mg/kg was selected for further studies and this dose was orally fed to mice for 3, 7 and 14 days to fix the duration of administration of NJE. Mice administered NJE for 7 days showed effective results by increasing the time spent on the open arm in EPM, an increased latency to enter the closed arm, an
increase in the number of transitions made between the open and closed arm and decreased the time spent on central zone and also increased the number of dips made into the open arms. This dosage also enhanced the time spent in the lit box of LDB and also elevated the number of licks made and shocks accepted in VCT. The brain monoamine and GABA neurotransmitter levels were elevated following this dosage and NJE at 250 mg/kg was also effective in modulating the levels of key antioxidant enzymes in the brain.

To elucidate the mode of anxiolytic action of NJE, the influence of GABA receptors in the anxiolytic actions of NJE was studied by co-administering NJE with antagonists of the GABA-receptor complex (picrotoxin and flumazenil) in mice. The results showed that treatment with these two antagonists significantly blocked the anxiolytic effects of NJE confirming NJE primarily and plausibly mediated alleviation of anxiety by activating the GABAergic receptor complex.

Oxidative stress (OS), of late has been implicated in the etiology of anxiety. OS was induced using BSO and as evidenced by the behavioural assays and by analysing the indices of OS viz. antioxidant enzymes, cortisol, acetyl choline, glutamate levels, MAO’s we saw an alleviation of OS induced anxiety in mice pre-fed with NJE/Dzp and over expression of two important biomarkers of OS induced anxiety, glyoxalase 1 and glutathione reductase 1 in BSO treated mice whose protein expression was attenuated in NJE and Dzp fed groups.

Thus, it could be appended that NJE exerts its anxiolytic actions by influencing the GABAergic transmission, as blocking of GABA\textsubscript{A} receptors by antagonists (picrotoxin and flumazenil) subsequently blocked the anxiolytic actions of NJE. A second mechanism may be that NJE was able to reduce MAO’s and enhance the monoamine and GABA levels. The roots showed enriched GABA and glutamate content which could be reaching the brain and enacting as a benzodiazepines, influencing NJE to exert its anxiolytic actions. NJE was able to alleviate OS induced anxiety mainly via its antioxidant machinery by elevating the levels of biologically important antioxidant enzymes and modulating the key protein biomarkers of OS induced anxiety viz. glyoxalase 1 and glutathione reductase 1. This property of NJE could also be attributable to the array of sesquiterpenes, polyphenols, melatonin
present in NJE which are acknowledged with scavenging ROS and exerting neuroprotectant properties.

The biodistribution and pharmacokinetics of NJE was studied in mice and rabbits. The identified metabolites (by LC-MS) were radiolabelled with technetium$^{99m}$ for analysing the blood kinetics and biodistribution pattern. Further, the metabolites reaching the brain were investigated using UPLC/MS. The pharmacokinetics parameters analysed showed that NJE had a $t_{1/2}$ of 6.56 h, Vd of 2.014 mL, $K_e$ of 0.105 h and total clearance of 0.212 mL/h. Following this, its biodistribution revealed maximum accumulation in the kidneys followed by the liver, lungs, intestine, bone, spleen. Very little activity was recorded from the brain (0.1 %) mainly because of penetrability of NJE into the complex blood brain barrier and solubility of the drug or may be this was sufficient enough for NJE to exert its actions. Hence, to further elucidate the mode of anxiolytic action, the metabolites of NJE crossing the blood brain barrier were studied and UPLC/MS identified zacopride hydrochloride (m/z 346) 1 h post administration, gansongone (m/z 218) and nardosinonediol (m/z 252), 1.5 h after administration of NJE, beta-ionone (m/z 192) & nardosinonediol (m/z 252), 2 h post administration, kanshone A (m/z 264), kanshone B (m/z 267) and Kanshone E (m/z 234), 4 h post administration. Zacopride hydrochloride, a benzamide is a 5-HT4 agonist and 5-HT3 antagonist and is attributed with anxiolytic properties. Beta-ionone, has been shown to enhance the antioxidant status and is also responsible for the pleasant odour of *Nardostachys jatamansi*.

Further, a nutraceutical with herbal additives of NJE was prepared and analysed for its physicochemical, microbiological and anxiolytic properties. The drink had an overall acceptability of 7.8 on a 9-point hedonic and was shelf stable for 6 months and aided in diminution of anxiety evidenced by behavioural parameters and also elevated the brain monoamine and GABA neurotransmitters. The drink on long term consumption promises to provide solace to anxious.

**Major findings of the Study**

- NJE at 250 mg/kg orally administered for seven days was effective in alleviating anxiety in mice and this dosage also elevated the brain GABA and monoamine neurotransmitter levels and enhanced the biological antioxidant enzymes
NJE exerts its anxiolytic actions by influencing the GABAergic transmission
NJE contains enriched GABA and glutamate content which may be reaching the brain and enacting as benzodiazepines influencing NJE to exert its anxiolytic actions
NJE attenuates OS induced anxiety via its antioxidant machinery (sesquiterpenes, polyphenols, flavonoids, and melatonin) by elevating the biological antioxidant enzymes and modulating key protein biomarkers of OS induced anxiety viz. glyoxalase 1 and glutathione reductase 1.
Pharmacokinetics study showed NJE to have $t_{1/2}$ of 6.56 h, Vd of 2.014 mL, Ke of 0.105 h and total clearance of 0.212 mL/h.
Biodistribution studies showed NJE to be majorly accumulating in the kidneys, followed by liver, intestine, bone and spleen with little activity recorded from the brain (0.1 %).
Metabolites of NJE crossing the complex blood brain barrier and contributing majorly to the anxiolytic effects of NJE were found to be zacopride hydrochloride, gansongone, nardosinonediol, beta-ionone, kanshone A kanshone B and kanshone E. Zacopride hydrochloride, a benzamide is a 5-HT4 agonist and 5-HT3 antagonist and is attributed with anxiolytic properties. Beta-ionone, has been shown to enhance the antioxidant status and inhibit lipid peroxidation.
Nutraceutical with herbal additives of *Nardostachys jatamansi* aided in alleviation of anxiety promising to provide solace to anxious on long term consumption.

**Implications of the study**

Major side-effects of the anxiolytic drugs have been listed. It is quite likely that an extract from a natural source given in the form of food might overcome or avoid drug dependency and many other side effects. The developed nutraceutical with herbal additives of *Nardostachys jatamansi* promises to attenuate anxiety and also provides general nutrition apart from relieving people of the stigma of consuming drugs.