Chapter-2

Review of Literature
2. Chapter-2, Review of Literature

2.1 Introduction to Nardostachys jatamansi DC / Valeriana jatamansi

*Nardostachys jatamansi DC* is well known medicinal herb. Botanical naming of *Nardostachys jatamansi DC* is interesting because of variation in species and place of origin. In 1790 Sir William Jones, famous orientalist, discovered that ‘Nardus’ of Greeks, the ‘Spikenard’ of the Holy bible, ‘Sumbul-e- Hind’ of Arabians and ‘Balchir’ of India are the ‘Jatamansi’ of Sanskrit (IUCN), Leaman 2007). Initially there was confusion in naming of species which De Candolle in 1821 described as *Valeriana jatamansi* and finally in 1830 as *Nardostachys jatamansi*. *Nardostachys jatamansi* is also now known as *Nardostachys grandiflora* (Houghton 1997) & (Duke 2007).

2.1.1 Scientific Classification of Nardostachys jatamansi DC/Valeriana jatamansi (VJ)

<table>
<thead>
<tr>
<th>Table 1: Scientific Classification</th>
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<td>Kingdom</td>
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<td>Binomial name</td>
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2.1.2 Ayurvedic Properties and Action (Health & Welfare n.d.)

Rasa : Tikta, Kasaya
Guna : Laghu
Virya : sita
Vipaka: Katu
Karma : Medhya, Tridoanut, Varnya, Nidrajanana, Kushaghna.
2.1.3 Synonyms of *Nardostachys jatamansi* DC

Table 2: Synonyms

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<thead>
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2.1.4 Occurrence and distribution

Jatamansi is distributed in sub-alpine to alpine regions at an altitude of 3000–5000 m in dry, open conifer forests, rocks, edges, small depressions, scrubs and in open meadows mostly on north facing stony and grassy slopes, and on the turf of glacial flats. It is found in the alpine areas of India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim), Pakistan, Nepal, Tibet, China and Yunan (Kokate et al. 1994) & (Polunin & Adam 1997).

Due to over-exploitation of the rhizome, the species has been listed in red data book as endangered in most of its natural populations and critically endangered in few other populations (MP & ARK. 1988).
2.1.5 Botany of *Nardostachys jatamansi* DC

![Plant and rhizome of Nardostachys jatamansi DC](image)

**Figure 2: Nardostachys jatamansi DC Plant and rhizome.**

Jatamansi is a perennial herb with a spindle-shaped woody, aromatic rootstock covered with short, thick, dark reddish brown thick fibers. It reaches the height of 10-60 cm. The fibers are either remnant of petioles of old leaves. It has single long tap root with rhizome 2-12 in numbers and flowering stems 5-30 cm. The leaves of Jatamansi are elliptic-lanceolate or spathulate, are long and narrow of about 5-20 cm mostly basal and arising from the stout and woody rootstock covered with dark fibers of old leaves. Flowers are rose-purple to whitish in appearance dense head borne in terminal. Calyx is coloured, 5-lobed; the lobes enlarge in fruit and become papery. Corolla tube is 6-20 mm long with 5-rounded spreading lobes. Fruit is small, 4 mm long, covered with minute hairs. It has been described as a combination of three tastes; bitter, astringent and sweet. The whole plant has a distinct lingering smell.

2.1.6 Medicinal properties

It is supposed to possess stimulant and antispasmodic properties. It is used in the treatment of epilepsy, hysteria, convulsive ailments, palpitations of the heart, diseases of the eye, itch, boils, swellings, diseases of the head, hiccup, etc. (Dweck, 1996). Other use includes ailments of the hair. The roots are also used for improving the complexion, increasing the luster of the eye and promoting the growth and increasing the blackness of
the hair (Jayaweera 1982). Jatamansi consists of dried rhizome of *Nardostachys jatamansi* DC. It has been used in herbal combinations with other herbs to evaluate depressant activity (Indurwade & Biyani 2000). According to Ayurveda the roots and rhizome of *Nardostachy jatamansi* DC. have various effects on ‘doshas’. The plant is Vatashamak by snigdha; pittashamak by sheeta, tikta, kashaya, madhur, kaphashamak by tikta and tikshna. Ultimately it is tridoshashamak but especially kapha-pittanashak been clinically employed for their anti-ischemic, antioxidant, anticonvulsant, and neuroprotective activities. *Nardostachy jatamansi* DC. also works as a memory enhancer. Furthermore, it also reversed aging-induced amnesia due to natural aging of mice. Rhizome of *Nardostachy jatamansi* DC is proved to be a useful memory restorative agent in the treatment of dementia and as anti-stress (Lyle, Gomes, et al. 2009).

### 2.1.7 Phytochemistry

The roots of the plant contain essential oil, rich in volatile constituents like sesquiterpenes and non-volatile constituents like sesquiterpenes, coumarins lignans, neolignans, alkaloids and steroids. Jatamansone or valeranone is the principal sesquiterpene. Other sesquiterpenes include nardostachone, dihydrojatamansin, jatamansinol, jatamansic acid, jatamansinone, jatamansinol, oroseolol, oroselone, seselin, valeranal, nardostachyin, nardosinone, spirojatamol, jatamol A and B, calarenol, seychellene, seychelane, coumarin: jatamansin or xanthogalin. A new sesquiterpene acid, nardin and new pyranocoumarin: 2, 2-dimethyl-3-methoxy-3, 4-dihydropyranocoumarin have been reported. Actinidine, an alkaloid has been reported (Gupta et al. 2012) & (Singh et al. 2009).
Structure of some of chemical constitutes

Table 3: Structure

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2.1.8 Medicinal Properties

a) Antioxidant activity

Nardostachys jatamansi induces a state of resistance against stress in organisms. An aqueous extract of Nardostachys jatamansi was used to investigate the antioxidant potential and anticataleptic agents by inducing catalepsy in rat by administering haloperidol in rat model. Nardostachys jatamansi reverses the haloperidol-induced catalepsy in rats and reduces oxidative stress (Habibur Rahman, Shaik, et al. 2011). Haloperidol induced (1 mg/kg, ip) catalepsy was effectively treated by median dose that is 250mg/kg while the results remain similar by increasing the dose up to 500 mg/kg. (Rasheed et al. 2010) The anti-peroxidative property of Jatamansi was investigated using an iron-induced lipid peroxidation model in rat liver, quantified by thiobarbituric acid reactive substance (TBARS) increased while glutathione (GSH) reduced significantly as a result of haloperidol administration. However, after their treatment with Nardostachys jatamansi, the substances were restored to near normal level. They have observed in their study that the extract provides protection against lipid peroxidation (Tripathi et al. 1996). Ethanolic extracts of Nardostachys jatamansi (NJE) were administered in Wister rats in two doses followed by immobilization stress on the 5th day. Rats that received different doses of NJE did not show clinical signs of toxicity and in vitro study showed free radical scavenging activity of NJE, as evidenced by low IC50 value. Pre-treatment with NJE-200 and 500 mg/kg significantly decreased lipid peroxidation (LPO) and nitrite level and increased the catalase activity in the brain. Presence of flavonoids and polyphenols is responsible for the antioxidant property of NJE and may also be responsible for its anti-stress effect (Lyle, Bhattacharyya, et al. 2009). Antioxidant potential and acetyl cholinesterase inhibition activity was done in sleep deprived amnesic rats by using methanolic extract of roots of Nardostachys jatamansi DC. This study demonstrated that NJ.Cr showed neuroprotective activity by inhibition of AChE and antioxidant activity which enhances memory like the synthetic drug Piracetam (H Rahman et al. 2011).
b) Hepatoprotective action

The root extract of *N. jatamansi* possess the hepatoprotective activities. A study reported that 50% ethanolic extract of the jatamansi rhizomes (800 mg/kg body wt) have hepatoprotective activity. Rats treated with the extract of jatamansi significantly improved the liver damage in rats exposed to the hepatotoxic compound thioacetamide. There were significantly reduced levels of serum transaminase and alkaline phosphatase in jatamansi pretreated rats was observed compared to thioacetamide alone treated group of animals. Increase in survival in rats was also reported when rats were pretreated with extract of jatamansi and further intoxicated with LD90 dose of the hepatotoxic drug (Ali et al. 2000).

c) Nootropic diseases

Three doses of the ethanolic extract of *N. jatamansi* were administered in both young and aged mice. The 200 mg/kg dose of the extract improved learning and memory effectively in young mice and reversed age related amnesia in older mice. The reversal of scopolamine- induced amnesia by NJE may be because of facilitation of cholinergic transmission in the brain. Hence, *N. jatamansi* could be a useful agent in restoring memory in case of dementia seen in elderly persons (Joshi & Parle 2006).

The administration of the ethanolic extract of *N. jatamansi* for 21 days elevated acetyl cholinesterase (AChE) activity levels in the frontal cortex thereby suggesting a possible mechanism for a nootropic effect of *N. jatamansi* (Karkada et al. 2012). In our study, we observed that MENJ (200 and 400mg/kg) has shown significant protection from loss of memory and cognition impairment due to sleep deprivation. At both dose levels MENJ showed decrease in Acetyl cholinesterase activity compared to sleep deprived animal. It suggests about its mechanism of memory enhancing by increasing cholinergic level (H Rahman et al. 2011).

d) Anticonvulsant activity

It has been reported that the the ethanol extract of the roots of *N. jatamansi* can be used to treat aniconvulsant activity. A study reported that 250 and 500 mg/kg of the root extract significantly reduced the extension/flexion ratio compared to control. A synergistic effect
was also observed for extract of *N. jatamansi* in combination with phenytoin increase in the seizure threshold by against maximal electroshock seizure model and exhibited minimal neurotoxicity against rota rod test. Thus, the effect of phenytoin alone and in combination with *N. jatamansi* extract is clearly demonstrated where concentration of phenytoin was very minimal (Rao et al. 2005).

e) **Cardio protective, hypolipidemic and respiratory disorder activity**

Limited data is available for cardiovascular activity. However few attempts have been made to elucidate the possible role of *N. jatamansi* in cardiovascular system and respiratory disorders. Studies have reported the role of *N. jatamansi* extract in cardio protective action. Doxorubicin is causing cumulative and dose dependent cardiomyopathy used to cause cardiac tissue damage especially on mitochondria and lysosome. Heart mitochondria isolated from rats treated with doxorubicin single dose single intraperitoneal injection of doxorubicin containing 15mg/kg was introduced and myocardial injury was induced. Effect of doxorubicin exhibited depressed rates of respiration, low respiratory control ratio (RCR), decreased Oxidative Phosphorylation ratio, Adenosine Triphosphate content and cytochromes. The doxorubicin given rats also showed significant changes in the lysosomal enzymes (Cathepsin-D, Acid phosphatase, BE- TA-D-glucoronidase, BETA-D-galactosidase and BETA-N-acetyl glucosaminidase) and membrane bound phosphatases. Also myocardial damage like loss of myofibrils, mitochondrial swelling, and cytoplasmic vacuolization were assessed by Transmission Electron Photomicrograph. Pretreatment with *N. jatamansi* 500mg/kg dose of for seven days ameliorated the observed abnormalities and significantly prevented the mitochondrial and lysosomal damage, membrane bound phosphatases and ultrastructural studies in caused by doxorubicin in rats. These reports also suggested that the cardioprotective efficacy of *N. jatamansi* may be because of its antioxidant effect as well as by the attenuation of the oxidative stress (Subashini et al. 2007).

*N. jatamansi* is very much used as a cardiotonic agent and in the management of respiratory disorder in the Unani system of medicine from very earlier, but no proper scientific data is available to support Ayurvedic claim. A study also reported that 50%
ethanolic extract *Curcuma longa* (tuber) and *N. jatamansi* (whole plant) have increased HDL/total Cholesterol ratio in triton induced dyslipidemic rats and reduce the ratio of total cholesterol to phospholipid ratio (Dixit et al. 1988).

f) **Antifungal and antibacterial activity**

Effect of *N. jatamansi* oil for its efficiency against *Aspergillus flavus, A. fumigatus, A. sulphureus, Mucor fragilis* and *Rhizopus stolonifer*. Depending upon the concentrations, the jatamansi oil was reported to be fungistatic or fungicidal to one or the other molds (Sarbhyo et al. 1978). In the study screening of antimicrobial action with the methanolic extract of *Nardostachys jatamansi* DC against *Saccharomyces cerevisiae, Aspergillus niger, Candida albicans, Streptococcus faecalis, Klebsiella pneumonia, Klebsiella pneumonia, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bordetella bronchiseptica, Bacillus subtilis, Bacillus pumilus,* and *Bacillus cereus varmycoides*. It was concluded in this study that *Nardostachys jatamansi* DC is effective against most of the microorganisms thereby justifying its role as antimicrobial and antifungal agent. Fungistatic spectrum of *Nardostachys jatamansi* DC evaluated and the results showed that the plant is effective against *Aspergillus flavus Aspergillus niger* and *Fusarium oxysporum* (Mishra et al. 1995).

g) **Effect on Estrogen and hair growth**

*Nardostachys jatamansi* DC is studied for the growth of hairs due to chemotherapy. Ethanolic extracts of *Nardostachys jatamansi* DC have shown hair growth promotion activities with looking at various parameters like hair density, lymphocyte count and testosterone level along with histo-pathological study. In next step hair growth study was design not only to see effect of extract on hair growth but also Hair growth initiation time was markedly reduced to half. Effects of isolated fraction named as Nardal, Jatamansic acid, Nardin was also checked for hair growth properties (Gottumukkala et al. 2011).
h) Hypoglycemic activity

In one study it was observed that effective dose for hypoglycemic activity of *Nardostachys jatamansi*. A study showed that 500mg/kg Hydroalcoholic extract of *Nardostachys jatamansi*. DC reduced the glucose level in Wistar albino normal rats, glucose loaded and alloxan induced diabetes (on day 15 and 30) rats as compared to respective control rats (Mahesh et al. 2008). Antidiabetic study was conducted by using 400mg/kg, 800mg/kg and 1200mg/kg dose for 10 days. Results depict that 1200mg/kg dose had significant antihyperglycemic effects as compared to disease model rats. Diabetic study was also confirmed by using STZ injection. Cytokines and STZ both cause damage to β cells which is protected by extract of NJ. Cr by inhibiting NF-κB activation, iNOS and NO production (Song et al. 2010).

i) Antidepressant activity

The extract of jatamansi has shown to have antidepressant activities. A study reported overall increase in the levels of central monoamines and inhibitory amino acids helps in treatment of depression, including a change in the levels of serotonin, 5-hydroxyindole acetic acid, gamma-amino butyric acid, and taurine Rat brain treated with root extract of *N. jatamansi* (Habibur Rahman 2010). One ,more study suggested that the antidepressant-like effect of the extract may also be due to interaction with GABA receptors, resulting in decrease in the levels of GABA in mouse brain. The extract have also decreased the whole brain MAO-A and MAO-B activities, thus increased the levels of monoamines. Swiss albino mice treated with extract of jatamansi produced significant antidepressant- like effect in both tail suspension and forced swim tests. The efficacy of the extract was found to be comparable to imipramine and sertraline (Dhingra & Goyal 2008).

j) Effect on insomnia

A polyherbal preparation containing *N. jatamansi* 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 (Usha Rani & Naidu 1998). MENJ at doses of 200 mg/kg and 400mg/kg treated groups showed a significant inhibition of AChE activity and improved antioxidants enzyme levels in sleep deprived amnesic mice. These findings
suggest MENJ exerts a protective effect against loss of memory and cognitive deficits due to its inhibition of AChE and protection from oxidative damage due to sleep deprivation (Habibur Rahman, P, et al. 2011).

### k) Anti-Parkinson activity

Parkinson's disease (PD) is very common and disease. Oxidative stress conditions have been evidenced in these neurodegenerative diseases. There are some reports which confirm the potential role of *N. jatamansi* to protect the brain damage. A study reported that that ethanol extract of *N. jatamansi* roots can restore the neuronal injury in caused by Parkinson's in rat in dose dependent manner (Ahmad et al. 2006). In another study Haloperidol was administered to rats to induce Parkinsonism. Treatment with hydro alcoholic root extract of *N. jatamansi* roots extract reversed the effect of Parkinsonism significantly, when compared to drugs antioxidant potential has contributed to the reduction in the oxidative stress and catalepsy induced by haloperidol administration (Rasheed et al. 2010).

A strong linkage between Insomnia and Alzheimer’s disease (AD) has been hypothesized. A study reported that the methanolic extract of *Nardostachys jatamansi* DC rhizome was investigated on sleep deprived amnesic mice in order to assess anti-amnesic activity. Pretreatment of animals with extracts of *Nardostachys jatamansi* DC (400 mg/ kg body weight) and for 14 days was after 5 days sleep deprivation showed considerable improvement in learning and cognition parameters in behavioral tests. The loss of memory and cognitive deficits due to sleep deprivation could be protected by methanolic extracts of *Nardostachys jatamansi* DC (Rahman & Muralidharan 2010)

One more study in which same experimental condition was s but treatment time was for 19 days. The mice brain was evaluated for Acetyl cholinesterase (AChE) activity, glutamate, antioxidants enzymes Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GRD), Glutathione peroxidase (GPx), Lipid peroxidase assay (LPO) and ascorbic acid (Vit.C) on the 19th day. Significant inhibition of AChE activity and improved antioxidants enzyme levels in sleep deprived amnesic mice was reported after treating the groups with MENJ doses. The protective effect of MENJ against loss of
memory and cognitive deficits due to its inhibition of AChE as well as protection from oxidative damage due to sleep deprivation is demonstrated by the above findings (H Rahman et al. 2011).

1) Role in nervous system

Jatamansi was used for treatment of this disease from long time in Indian system. The cholinergic hypothesis of Alzheimer's disease (AD) has provided the rationale for the current pharmacotherapy of this disease, in an attempt to reduce the cognitive decline caused by cholinergic deficits (Singh et al. 2013). Only a limited number of drugs are commercially available. The search for potent and long acting acetyl cholinesterase (AChE) inhibitors that with minimal side effects in AD patients is still ongoing. A study reports 50% inhibition of AChE activity caused by methanolic extracts of *N. jatamansi* treatment. Along with AChE activity extracts have also been reported for neurotrophic effects and for improving cognition (Vinutha et al. 2007).
2.2 Introduction to Valeriana wallichii DC

Valeriana jatamansi Jones (syn. V. wallichii DC) has been used therapeutically in Indian Ayurvedic, Tibetan Buddhist and traditional Chinese schools of medicine for many centuries. Indian Valeriana V. wallichii DC has similar effect like European valerian, V. officinalis. Valerian is recommended for treating restlessness and sleep disorders, skin problem, analgesic and against rotavirus, based on scientific studies.

2.2.1 Scientific Classification of Valeriana wallichii DC

Table 4: Scientific Classification

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2.2.2 Ayurvedic property and actions (Health & Welfare n.d.)

Rasa : Katu, Tikta, Kasaya
Guna : Laghu, Snigdha
Virya : Usna
Vipaka : Katu
Karma : Visaghna, Tridosahara, Raktadosahara, Manasadoshara.

2.2.3 Occurrence and distribution (Singhal & Neetu 2013)

Tagara consists of predominantly dried rhizome, stolon and small portion of root of Valeriana wallichii DC, (Fam.Valerianaceae): a hairy perennial herb, growing in temperate d Himalayas from Kashmir to Bhutan and Khasia hills up to an altitude of 3,000 m. Valeriana wallichii is an extremely polymorphous complex of sub-species with natural populations dispersed throughout temperate and sub-polar Eurasian zones. The species is common in damp woods, ditches, and along streams in Europe, and is cultivated as a
medicinal plant, especially in Belgium, England, Eastern Europe, France, Germany, India, the Netherlands, the Russian Federation, and the United States of America.

### 2.2.4 Synonyms of *Valeriana wallichii* DC

**Table 5: Synonyms**

<table>
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### 2.2.5 Botany of *Valeriana wallichii* DC

**Figure 3: *Valeriana wallichii* DC. Plants and Rhizome**
The plant herb up to 45 cm in height, root occurs in short thick, irregular pieces about 5 cm long and 2.5-2.75 cm in diameters. Roots are marked with transverse ridges and bearing numerous, prominent, circular tubercles, to some of which on the under surface, thick rootlets are attached. The upper surface bears the remains of leaves. The rhizome is hard and tough internally, it is greenish-brown in color. The odour is powerfully valerianaceous. Flowers of valerian are often deciduous, white to a pink, in terminal corymbs and unisexual, male and female in different plants.

2.2.6 Phytochemicals

The roots of *Valeriana wallichii* DC contain volatile oils, iridoïds, alkaloids and flavonoids. The oil contains sequiterpena, valeric acid, terpene alcohol, bornyl esters, of formic acid, camphene, terineol, sesquiterpenoids, iridoïds (valepotriates), flavonoids, and lignans are major components (Sah Pilkhwal et al. 2010). A study on essential oil of *Valeriana wallichii* DC reported total of 27 components which were accounting for 94.8% of the total oil. And 7% of oil was Monoterpenoids containing 8 components. And 27 components were sisquiterpenoids. Major constituents identified were patchoul, α-bulnesene (13.8%), isovaleric acid (12.9%), α-guaiene (8.7%), and 3-methylvaleric acid (Liu et al. 2013).

2.2.7 Medicinal properties

a) Anxiolytic, stress and Antidepressant Effects

There are many studies supporting anxiolytic properties of *Valeriana jatamansi*. A clinical study reported that administration of with *Valeriana jatamansi* regularly for two months not only reduced stress, attenuated anxiety, negated depression but also enhanced adjustment bwithout altering memory, attention and concentration in human. The study also supported that *Valeriana wallichii* may be a safer alternative to benzodiazepines for the therapy of stress related clinical disorders (Bhattacharyya et al. 2007).

Another study reported anxiolytic effect of the 45% methanolic and 35% ethanolic extract (patented special extract phytofin Valerian 368) a dose range of 100-500 mg/kg bw.
Antidepressant activity was also found from its primary extract (35% ethanolic extract) phytofin Valerian 368 after subacute treatment (Hattesohl et al. 2008).

One more clinical study forty healthy volunteers were done. It was double blind study in which either 100 mg of valerian extract, 209 mg of propanolol, or a combination of both was given to volunteers. In contrast to propanolol, valerian was not associated with reduction in physiological arousal under stress but it did show improvement in anxiety and mood (Kohnen & Oswald 1988).

b) Effect on sleep

A study was performed on sleep-wake profile and level of brain monoamines on Sprague Dawley rats. aqueous root extract of of 100, 200 and 300 mg/kg body weight Valeriana Wallichii was administration to rats and electrodes and transmitters were implanted to record EEG and EMG in freely moving condition and the changes were recorded telemetrically. Duration of NREM sleep as well as duration of total sleep was increased significantly while sleep latency was significantly decreased after treatment with Valeriana Wallichii at the doses of 200 and 300 mg/kg with control. Treated animals also showed increased EEG slow wave activity during NREM sleep at the doses of 200 and 300 mg/kg. Level of biological amines like norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and hydroxy indole acetic acid (HIAA) were measured in frontal cortex and brain stem after VW treatment at the dose of 200mg/kg. It was found that NE and 5HT level were decreased significantly in both frontal cortex and brain stem. DA and HIAA level significantly decreased only in cortex. DOPAC level was not changed in any brain region studied. The study indicates that Valeriana Wallichii water extract has a sleep quality improving effect which may be dependent upon levels of monoamines in cortex and brainstem (Sahu et al. 2012).

c) Microbiological Studies

The antimicrobial (antifungal and antibacterial) and anti-inflammatory properties of Valeriana Wallichii was reported in the study. Chloroform fraction Valeriana Wallichii and hexane fraction Valeriana Wallichii exhibited significant activity against S. aureus
and *B. subtilis*, respectively. The chloroform fraction showed significant activity against *S. aureus* with 0.27 mg/ml MIC, where 0.31 mg/ml MIC was deduced for VW-3 fraction against *B. subtilis* hexane fraction was also found to be the most potent inhibitor of *M. canis*, showing 70% inhibition with an MIC value of 0.19 mg/ml. Considerable inhibitory activity was also observed for chloroform and water fraction against *M. canis* and *A. flavus*. A remarkable anti-inflammatory like activity was observed for the crude extract at a dose of 200 mg/kg at all observed durations. The result concluded that *Valeriana Wallichii* may be a potential source for activity guided isolation of natural products with antimicrobial and anti-inflammatory-like properties (Khuda et al. 2012).

d) **Effect on cognitive performance**

A study reported improved cognitive performance after treatment with *Valeriana wallichii*. Assessment parameters included scale of wellbeing and objective of cognitive and psychomotor performance and evaluation of the tolerance. The study was double blind with healthy subjects were treated with valerian syrup, tablets containing valerian and hops, flunitrazepam or a placebo. Next morning following treatment, impaired performance was observed in flunitrazepam group, where as those receiving valerian formulation noted feeling better, more alert and active on both subjective and objective ratings (Gerhard et al. 1996).

e) **Effect on GABAA receptors**

Effect of valerenic acid which is important constituent of *Valeriana wallichii* on GABA receptor was reported in a study. Effect of Valerian extracts was investigated using the two-microelectrode voltage clamp technique was used to measure chloride reflux through GABAA receptors. A polar extracts induced a significant enhancement of GABA, whereas polar extracts showed no effect. These results were confirmed by first using valerenic acid rich fractions and removal of sesquiterpenic acids from the ethyl acetate extract. The fractions with high contents of valerenic acid exhibited strong receptor activation and
removal of led to a loss of GABA enhancement. Which concluded that the extent of GABAA receptor modulation by is related to valerenic acid (Trauner et al. 2008).

One more study indicated active compounds 6-methylapigenin that was isolated from the rhizomes and roots of Valeriana wallichii DC and their identification was done using UV, NMR and mass spectral. Which was suggested to have possible agonistic properties (Wasowski et al. 2002).

The molecular was analyzed on the basis of Valerenic Acid action on GABA (A) receptors. Eport described a subunit specific modulation of GABAA receptors by VA (b3>b2>>b1-containing receptors. Positive allosteric modulation induced by a Valerenic Acid increased in the GABA sensitivity. At high concentrations, VA activates GABAA channels directly and also blocks the channel. The study suggested that sedative, hypnotic and anxiolytic effects with Valerian may be caused by interaction of VA with GABAA channels (Khom et al. 2007).

f) Hepatoprotective activity

Hepatoprotective activity of Valeriana Wallichii was concluded in a report in which the hepatotoxocities in animals were induced by CCl4. The animals were sacrifice on 8 days and blood was collected for biochemical analysis (aspartate aminotransferase [AST], alanine transaminase (ALT) and alkaline phosphatase). Liver tissue was extracted for histopathological examination and in vivo antioxidant tests Catalase [CAT], glutathione and malondialdehyde. Hepatotoxicity induced animal received 300 mg and 500 mg/kg body wt. aqueous extract of roots of Valeriana Wallichii The test extracts in the dose of 500 mg/kg were shown a significant decrease in the levels of AST and ALT (p>0.05) and CAT activity. 300 mg/kg dose of extract showed minimal hepatoprotection (Shariq Naeem Syed 2014).

g) Gastrointestinal and Cardiovascular disorder

On the basis of folkloric uses effect of crude extract of Valeriana wallichii rhizome and its fractions on antispasmodic and blood pressure lowering activities was studied. In rabbit jejunum preparations extract of Valeriana wallichii (0.1-3.0 mg/mL) caused relaxation of
spontaneous contractions. When tested against high K (+) (80 mM)-induced contractions it produced weak inhibitory effect, while caused complete relaxation of the contractions induced by low K(+) (20 mM). In the presence of glibenclamide (3 microM), the inhibitory effect of low K (+) was shifted to the right, similar to that produced by cromakalim while, verapamil caused no differentiation in its inhibitory effect against low and high K (+)-induced contractions. In guinea pig ileum, the plant extract produced similar results as in rabbit jejunum. Intravenous administration of Valeriana Wallichii Cr, produced fall in arterial blood pressure in normotensive anaesthetized rats and this effect was partially blocked by glibenclamide. In rabbit aortic preparations, plant extract also caused selective and glibenclamide plant extract also caused selective and glibenclamide-sensitive relaxation of low K (+) (20 mM)-induced contractions. Activity-directed fractionation studies revealed that the observed activity was distributed both in the chloroform and aqueous fractions. These results indicate that the antispasmodic and hypotensive effects of Valeriana wallichii are mediated possibly through K(ATP) channel activation, which justify its use in gastrointestinal and cardiovascular disorder (Gilani et al. 2005).

2.3 Introduction to Withania somnifera Linn

Withania somnifera, also commonly known as Ashwagandha, it is also known as Indian ginseng and winter cherry, has been an important herb in the ayurvedic and indigenous medical systems. Ashwagandha or Withania somnifera (family: Solanaceae) is found to be of great help in number of ailments like anxiety, depression, psychiatric disorders, female disorders, cough, rheumatism etc (kirtikar et al. 1975). The main constituents are alkaloids and steroidal lactones (Ray AB & M 1994). The biological activities of Withanolides (steroidal lactones), especially of the dominant Withanolide A and withaferin A, have been studied extensively and, more recently, have been shown to have anti-cancerous activity (Jayaprakasam et al. 2003). Withanolide A is having high medicinal value is known as important secondary metabolite of Withania somnifera, which includes potent anti-tumor and antioxidant properties and neurological disorder (Praveen et al. 2010) & (Patil et al. 2013). Withaferin A has been reported as promising anti-cancer drug candidate
due to its cytotoxic (Yousuf et al. 2011), apoptotic (Mayola et al. 2011) anti-metastatic (Thaiparambil et al. 2011), anti-mitotic (Stan et al. 2008) and anti-angiogenesis properties (Mohan et al. 2004). Withaferin A was reported for anti-cancer (3.5 mg/kg), anti-inflammatory, anti-parasitic (Kushwaha et al. 2012) and hepatoprotective (Sudhir & Budhiraja 1992).

2.3.1 Scientific Classification of *Withania somnifera* Linn

Table 6: Scientific Classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
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<tbody>
<tr>
<td>Division</td>
<td>Mangnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Mangnoliophyta</td>
</tr>
<tr>
<td>Order</td>
<td>Solanales</td>
</tr>
<tr>
<td>Family</td>
<td>Solanaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Withania</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>W. somnifera</em></td>
</tr>
</tbody>
</table>

2.3.2 Ayurvedic properties and action (Health & Welfare n.d.)

Rasa: Tikta, Kasaya
Guna: Laghu
Virya: Usna
Vipaka: Madhura
Karma: Rasayana, Vatakaphapaha, Balya, Vajikarana

2.3.3 Synonyms of *Withania somnifera* Linn

Table 7: Synonyms

<table>
<thead>
<tr>
<th>Sanskrit</th>
<th>Hayagandha, Vajigandha</th>
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<tbody>
<tr>
<td>Assamese</td>
<td>Ashvagandha</td>
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<tr>
<td>Bengali</td>
<td>Ashvagandha</td>
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<tr>
<td>English</td>
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</table>
2.3.4 Occurrence and distribution

*Withania somnifera* is found in subtropical and dried regions. It is commonly found in India, Pakistan, Afghanistan, Philistine, Egypt, Jordan, Morocco, Sri Lanka, Spain, Canary Island, Eastern Africa, Congo, Madagascar and South Africa. In India, *Withania somnifera* is widely distributed in north-western region, Maharashtra, Gujarat, Rajasthan, Madhya Pradesh, Orissa, Uttar Pradesh, Punjab plains extending to the mountain regions of Punjab, Himachal Pradesh and Jammu.

2.3.5 Botany of *Withania somnifera* Linn

![Withania somnifera Linn. Plant and Rhizome](image)

*Figure 4: Withania somnifera Linn. Plant and Rhizome*
Elongated, unbranched, varying thickness, roots bearing secondary root, buff to yellow in color, nodes prominent only on the side from where petiole arises, fracture; short and uneven, odor; characteristic, taste; bitter and acrid. It has sessile, axillary, greenish or lurid yellow flowers. They are hermaphrodite (has both male and female organs). The fruit is Orange-red berry, smooth, oblong, rounded or somewhat produced at base.

2.3.6 Phytochemistry (RP & BN. 1998) & (Padmawar n.d.)

The root *Withania somnifera* have been studied extracted, and isolated for phytoconstituents and twelve alkaloids, 35 withanolides, and several sitoindosides from *Withania somnifera* have been isolated and studied. The biologically active chemical constituents are alkaloids (withanine, somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-α-loyloxytropane, choline, cuscohygrine, isopelletierine, anaferine andanahydrine erine), steroidal lactones (withanolides, withaferins) whichaccount for its extraordinary medicinal properties, saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X). *Withania somnifera* is also rich in iron. *Withania somnifera* is also rich in iron.

<table>
<thead>
<tr>
<th>Table 8: Phyto constituents</th>
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<tbody>
<tr>
<td>Withanone</td>
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<tr>
<td>Withanolide D</td>
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<tr>
<td>Withanolide A</td>
</tr>
<tr>
<td>Withaferin A</td>
</tr>
</tbody>
</table>
2.3.7 Medicinal properties

a) Anti-inflammatory Properties

The effectiveness of Ashwagandha in a variety of rheumatologic conditions may be due in part to its anti-inflammatory properties, which have been studied by several authors. In a study powdered roots of *Withania somnifera* (1 g/kg suspended in 2% gum acacia, 50 mg/mL) was given orally one hour before the induction of inflammation by injection of Freund’s complete adjuvant in rats and continued daily for three days; phenylbutazone (100mg/kg) was given as a positive control. WS was found to cause considerable reduction in inflammation. Serum proteins (α2-glycoprotein, major acute phase α1-protein, and pre-albumin) was also determined and it was found to be in undetectable levels in the *Withania somnifera* treated group while it was considerable increase in the α2-glycoprotein in both arthritic and healthy rats. The study showed that symptoms of arthritis and other inflammatory conditions can be treated by *Withania somnifera* (Anbalagan & Sadique 1981).

A study has also shown to exert significant inhibitory effect on incorporation of 35S into the granulation tissue. The uncoupling effect on oxidative phosphorylation (ADP/O ratio reduction) was also observed in the mitochondria of granulation tissue. Further, Mg2+ dependent ATPase activity was found to be influenced by W. somnifera. W. somnifera also reduced the succinate dehydrogenase enzyme activity in the mitochondria of granulation tissue (Begum & Sadique 1987).

In another study on rats administered with *Withania somnifera* root powder 500, 1000, 1500, or 1200 mg/kg 3-4 hours prior to induction of inflammation caused dose-dependent suppression of α2-macro globulin which is an indicator for anti-inflammatory drugs in the serum. Maximum effect (about 75%) was seen at 1000 mg/kg (Begum & Sadique 1987).

One more study on long term administration of root powder of *Withania somnifera* (1000 mg/ kg, orally daily for 15 days) on paw swelling and bony degenerative changes in Freund’s adjuvant induced arthritis in rats was done. It was reported *Withania somnifera* caused significant reduction in both paw swelling and degenerative changes as observed by radiological examination (Begum & Sadique 1988).
b) Antitumor Properties

Effect of *Withania somnifera* cancer, the antitumor and radio sensitizing effects of WS have also been reported. In one study, WS was evaluated for its anti-tumor effect in urethane-induced lung adenomas in adult male albino mice. Simultaneous administration of *Withania somnifera* (ethanol extract of whole plant, 200 mg/kg daily orally for seven months) reduced tumor incidence significantly. The histological appearance of the lungs of animals protected by *Withania somnifera* was similar to those of control animals. In addition to providing protection from carcinogenic effects(Singh et al. 1986). One more study showed the growth inhibitory effect of *Withania somnifera* root ethanol extract (400 mg/kg for 15 days) in Sarcoma 180 (S-180), a transplantable mouse tumor. After intradermal inoculation of 5x10^5 cells of S-180 in BALB/c mice produced complete regression of tumor after the initial growth. A 55-percent complete regression was obtained at 1000 mg/kg(Devi et al. 1992). Antitumor and radio sensitizing effects of withaferin (a steroidal lactone of WS) were also seen in mouse Ehrlich ascites carcinoma *in vivo*.15 Withaferin A from *Withania somnifera* gave a radio sensitizer ratio of 1:5 for *in vitro* cell killing of V79 Chinese hamster cell at a non-toxic concentration of about 2 mM/L (Devi 1996).

c) Anti-stress Effect

To evaluate the anti-stress effect of *Withania somnifera*, an alcohol extract from defatted seeds of *Withania somnifera* dissolved in normal saline was given (100 mg/ kg i.p as a single dose) to 20-25g mice in a swimming performance test in water at 28º-30ºC. Controls were given saline. The extracts approximately doubled the swimming time when compared to controls(Singh et al. 1982). Glycosides of *Withania somnifera* (sitoindosides VII and VIII, 50 to 100 mg/kg) exhibited significant antistress activity in forced swimming induced immobility in mice, restraint stress induced gastric ulcers in rats, restraint-induced auto-analgesia in rats, restraint stress effect on thermic response of morphine in rats, and morphine-induced toxicity in aggregated mice(Bhattacharya et al. 1987). The mild unpredictable footshock stress procedure administered once daily for 21 days to adult male Wistar rats. Chronic stress induced significant hyperglycaemia, glucose intolerance increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression. These chronic
stress induced perturbations were attenuated by *Withania somnifera* (25 and 50 mg/kg b.w.) administered 1 h before foot shock for 21 days (Bhattacharya & Muruganandam 2003).

d) **Antioxidant Effect**

Antioxidant activity was checked by using component of *Withania somnifera* glycowithanolides of (10 or 20 mg/kg intraperitoneally once daily for 21 days) to rats. Dose-dependent increases in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration (2 g/kg/day intraperitoneally). Which indicated that *Withania somnifera* does have an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties (Bhattacharya et al. 1997). In another study, an aqueous suspension of WS root extract was evaluated for its effect on stress-induced lipid peroxidation (LPO) in mice and rabbits. LPO blood levels were increased by IV administration of 0.2 mg/ *siella pneumoniae* and 100 mg/kg of peptidoglycans (PGN) from *Staphylococcus aureus*. Simultaneous oral administration of *Withania somnifera* extract (100 mg/kg) prevented an increase in LPO. The result indicated that the almost innocuous doses of LPS and PGN used in this study that induced elevated levels of LPO were comparable to a mild bacteremia which may follow tooth extraction, streptococcal angina, etc (Dhuley 1998).

e) **Immunomodulatory Properties**

Use of *Withania somnifera* as immunobooster was also supported by studies. In a study Immunomodulatory and central nervous system effects (antistress, memory, and learning) in Swiss mice (15-25 g, 5-6 months old) and Wistar strain albino rats (120-150 g and 250-300 g) was studied on Glycowithanolides and a mixture of sitoindosides IX and X isolated from *Withania somnifera* were evaluated (Ghosal et al. 1989). The two compounds, in doses of 100–400 μg/mouse, produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes secreted by the activated macrophages. Both these compounds (50–200 mg/kg p.o.) also produced significant anti-stress activity in albino mice and rats and augmented learning
acquisition and memory retention in both young and old rats. These findings are consistent with the use of W. somnifera, in Ayurveda, to attenuate cerebral function deficits in the geriatric population and to provide non-specific host defense (Ghosal et al. 1989).

Root extract of WS was tested for immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone. Significant increases (p<0.05) in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in WS-treated mice compared to untreated control mice. The authors also reported significant increases in hemolytic antibody responses toward human erythrocytes which indicated immunostimulatory activity (Ziauddin et al. 1996).

f) Hemopoetic Effect

Withania somnifera helps in stimulation of stem cell proliferation which may further prove useful in cancer chemotherapy. Administration of WS extract was found to significantly reduce leukopenia induced by cyclophosphamide (CTX) treatment in Swiss albino mice. Total white blood cell count on the 12th day of the CTX-treated group was 3720/mm³; that of the CTX-plus- Withania somnifera group was 6120/mm³. In the CTX-plus-WS mice, the cellularity of the bone marrow was significantly increased (13.1 x 10⁶ /femur) (p<0.001) compared to the CTX-alone treated group (8 x 10⁶/femur). Similarly, the number of alpha-esterase positive cells (1130/4000 cells) in the bone marrow of the CTX-plus- Withania somnifera mice increased compared to the CTX alone mice (687/4000 cells) (Davis & Kuttan 1998) & (Ghosal et al. 1989).

g) Nervous System Effects

Total alkaloid extract, Ashwagandha (AG) of Withania somnifera roots has been studied for its effects on the central nervous system. AG exhibited a taming effect and a mild depressant (tranquilizer) effect on the central nervous system in monkeys, cats, dogs, albino rats, and mice. AG had no analgesic activity in rats but increased Metrazol toxicity in rats and mice, amphetamine toxicity in mice, and produced hypothermia in mice. It also potentiated barbiturate, ethanol, and urethane induced hypnosis in mice (Malhotra et al.)
1965). Effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extract of roots of cultivated varieties of *Withania somnifera* were studied on brain cholinergic, glutamatergic and GABAergic receptors in male Wistar rats. The compounds slightly enhanced acetylcholinesterase (AChE) activity in the lateral septum and globus pallidus, and decreased AChE activity in the vertical diagonal band. These changes were accompanied by enhanced M1-muscarinic-cholinergic receptor-binding in lateral and medial septum as well as in frontal cortices, whereas the M2-muscarinic receptor-binding sites were increased in a number of cortical regions including cingulate, frontal, piriform, parietal, and retropinal cortex. The data suggest the compounds preferentially affect events in the cortical and basal forebrain cholinergic-signal transduction cascade (Schlibes et al. 1997).

**h) Effects on the Endocrine System**

Based on the observations that *Withania somnifera* provides protection from free radical damage in the mouse liver, studies were conducted to determine the efficacy of WS in regulating thyroid function. WS significantly reduced hepatic lipid peroxidation and increased the activity of superoxide dismutase and catalase. The results suggest *Withania somnifera* stimulates thyroidal activity and also promotes hepatic antioxidant activity (Panda & Kar 1998).

**i) Effects on the Cardiovascular and Respiratory System**

The effect of *Withania somnifera* was studied on the cardiovascular and respiratory systems in dogs and frogs. The alkaloids had a prolonged hypotensive, bradycardiac, and respiratory-stimulant action in dogs. The pharmacological actions of the total extract of *Withania somnifera* roots on the cardiovascular and respiratory systems appeared to be due to its alkaloid content. The total alkaloids were more than twice as active as the 70-percent alcohol extract of *Withania somnifera* root. These studies were found to be consistent with the use of *Withania somnifera* as a tranquilizing agent (Malhotra et al. 1961).
2.4 Sleep Disorder

Sleep is a complex neurological process that is important in mammalian homeostasis, and required for survival. Duration of time required for normal function for human being varies adults need 6.5-8 h of sleep a day, teenagers sleeps for 9 hours while Infants sleep sleeps for 16 hours a day and older person sleep for very short duration (Lorton et al. 2006).

In humans, sleep is vital to maintain health and well-being due to its primary function of providing rest and restoring the body’s energy level. Earlier theories of sleep presumed that sleep occurred at the level of the whole organism and that sleep was governed by central control mechanisms. However, evidence now indicates that sleep might be regulated at a more local level within the brain: it seems to be a fundamental property of neuronal networks and is dependent on prior activity within each network (Krueger et al. 2009). Sleep sustains physical and cognitive performance, the immune system, stable mood, productivity, and quality of life. Sleep is necessary for health as its loss or restriction is associated with multiple detrimental consequences (Imeri & Opp 2009).

Sleeping disorders is a medical disorder of the sleep patterns of a person or animal. Some sleep disorders are serious enough to interfere with normal physical, mental and emotional functioning(wikipedia.org, 2014). Disturbed sleep includes the inability to fall asleep, the inability to go back to sleep, and frequent waking up during the night. Sleep disorders can make you feel tired, fatigued, and irritable, altered concentration, impair of cognitive and psychological functioning and worsen physical health (Brand & Kirov 2011) & (Roddick n.d.). A neurological disorder marked by a sudden recurrent uncontrollable compulsion to sleep.
2.5 Incidence of Sleep Disorders

According to World Association of Sleep Medicine, sleep problems constitute a global epidemic affecting up to 45% of the world’s population (WASM 2014). Nearly 5% Indians aged 50 years and above are suffering from sleep disorders where Indian women (6.5%) outnumber men (4.3%). The findings suggest that sleeplessness epidemic affects an estimated 150 million in developing world (Sinha 2012).

The Report from Ghana and the University of the Witwatersrand in South Africa on the sleep quality of 24,434 women and 19,501 men aged 50 years mentioned about sleep problem in eight locations in rural populations in Ghana, Tanzania, South Africa, India, Bangladesh, Vietnam and Indonesia, and an urban area in Kenya. There was striking variation across the countries surveyed – Bangladesh, South Africa and Vietnam had extremely high levels of sleep problems, in some cases surpassing Western sleeplessness rates.

![Figure 5: Incidence of Sleep disorder](image)

However India and Indonesia reported relatively low levels of severe sleep problems. The research also found a higher prevalence of sleep problems in women and older age groups, consistent with patterns found in higher income countries (WARWICK n.d.).
insomnia is more common in women, with a female-to-male ratio of 3:2. Hormonal variations during the menstrual cycle or during menopause may cause disruptions in sleep. Obstructive sleep apnea (OSA) is more common in men (4%) than in women (2.5%). More than 30% of adults over the age of 50 have some type of sleep disorder. People who are elderly experience a decrease in total sleep time, with more frequent awakenings during the night. Elderly persons also have a higher incidence of general medical conditions and are more likely to be taking medications that cause sleep disruption. People who are elderly may have widespread or multisite pain that is associated with sleep difficulty, according to the Maintenance of Balance, Independent Living, Intellect, and Zest in the Elderly study (MOBILIZE) study (Chen et al. 2011).

2.6 Importance of sleep

Sleep is very much important as it is important for own metabolic functions and health benefits. Below are the various benefits of sleep (Publications 2006).

Learning and memory: Sleep helps the brain commit new information to memory through a process called memory consolidation. In studies, people who’d slept after learning a task did better on tests later.

Metabolism and weight: Chronic sleep deprivation may cause weight gain by affecting the way our bodies process and store carbohydrates, and by altering levels of hormones that affect our appetite.

Safety: Sleep debt contributes to a greater tendency to fall asleep during the daytime. These lapses may cause falls and mistakes such as medical errors, air traffic mishaps, and road accidents.

Mood: Sleep loss may result in irritability, impatience, inability to concentrate, and moodiness. Too little sleep can also leave you too tired to do the things you like to do.

Cardiovascular health: Serious sleep disorders have been linked to hypertension, increased stress hormone levels, and irregular heartbeat.
**Disease:** Sleep deprivation alters immune function, including the activity of the body’s killer cells. Keeping up with sleep may also help fight cancer.

**Repairs cells:** Sleep slows metabolism, heartbeat and breathing rate, which helps the body replenish after daily physical activity.

**Releases growth hormones in young adults:** Some growth hormones are released only during sleeping state. This hormone aids the growth of bones, tissues and new red blood cells.

**Strengthens our immune System:** In sleep deprived condition T-cells go down, and inflammatory cytokines go up.

**Strengthens our nervous systems:** Sleep helps to reduce stress level and improve cognitive functions.

### 2.7 Etiology and Types of Sleep Disorders

There are more than 70 different sleep disorders, which are generally classified into one of three categories: (NSF, 2014) & (Melinda Smith et al. 2013)

A. Lack of sleep (e.g., insomnia),  
B. Disturbed sleep (e.g., sleep apnea, REM sleep behavior disorder, restless leg syndrome and periodic limb movement disorder)),  
C. Excessive sleep (e.g., narcolepsy).

A. Lack of sleep

Primary insomnia: Chronic difficulty in falling asleep and/or maintaining sleep. Insomnia is the inability to fall asleep. It is a common sleep problem that most people experience at least occasionally. When it occurs, people feel tired much of the time and often worry a lot about not getting enough sleep. Consequently, insomnia often disrupts daily life.
Insomnia can result from the following:

- Diet (e.g., intake of caffeine or alcohol)
- Emotional difficulties
- Stress
- Underlying disease

For short-term insomnia, sleeping pills can be effective. For long-term insomnia, however, sleeping pills can actually worsen the condition.

**B. Disturbed sleep**

1. **Bruxism**
   Involutarily grinding or clenching of the teeth while sleeping.

2. **Delayed sleep phase syndrome (DSPS)**
   Inability to awaken and fall asleep at socially acceptable times, but no problem with sleep maintenance, a disorder of circadian rhythms. Other such disorders are advanced sleep phase syndrome (ASPS) and Non-24-hour sleep-wake syndrome (Non-24), both much less common than DSPS.

3. **Hypopnea syndrome**
   Abnormally shallow breathing or slow respiratory rate while sleeping.

4. **Cataplexy**
   A sudden weakness in the motor muscles that can result in collapse to the floor.

5. **Night terror**
   Pavor nocturnus, sleep terror disorder it abrupt awakening from sleep with behavior consistent with terror.

6. **Parasomnias**
   Disruptive sleep-related events involving inappropriate actions during sleep stages - sleep walking and night-terrors are examples.

7. **Rapid eye movement behavior disorder (RBD)**
   Its acting out violent or dramatic dreams while in REM sleep. In a person with REM sleep behavior disorder, these signals translate into images that make up dreams. If
the signals are interfered with, the person may physically act out dreams during sleep. For example, if a patient with REM sleep behavior disorder dreams about running, he or she might actually get up and run.

8. Restless legs syndrome (RLS)

Restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) are common sleep disorders, especially in the elderly. RLS is a genetic disorder resulting in prickly or tingling sensations in the leg that cause patients to want to move their legs. It often results in insomnia. PLMD causes jerking in the legs or arms that occurs frequently during resting or sleeping. Jerking may occur as many as 3 times in a minute and each jerk can wake the patient. An irresistible urge to move legs. RLS sufferers often also have PLMD.

9. Situational circadian rhythm sleep disorders

Shift work sleep disorder (SWSD) and jet lag.

10. Obstructive sleep apnea

Obstruction of the airway during sleep, causing lack of sufficient deep sleep; often accompanied by snoring. Other forms of sleep apnea are less common.

11. Sleepwalking or somnambulism

Engaging in activities that are normally associated with wakefulness (such as eating or dressing), which may include walking, without the conscious knowledge of the subject.

12. Nocturia

Under this condition there is frequent need to get up and go to the bathroom to urinate at night. It differs from Enuresis, or bed-wetting, in which the person does not arouse from sleep, but the bladder nevertheless empties.

13. Somniphobia

A dread of sleep.

C. Excessive sleep

1. Narcolepsy

Narcolepsy is the only major sleep disorder with a known genetic cause. Excessive daytime sleepiness (EDS) often culminating in falling asleep spontaneously but unwillingly at inappropriate times it is a condition that causes patients to fall asleep
uncontrollably throughout the day for periods lasting less than a minute to more than half an hour. During sleep, narcoleptics have an abnormal sleep pattern. Narcolepsy usually is a genetic (inherited) disorder, although it may be associated with brain damage or neurological disease.

2. Cataplexy

It is weakness or paralysis of the muscles. In narcoleptic patients, it may be triggered by tiredness and intense emotions and may be accompanied by short, sudden episodes of laughter or anger. When cataplexy occurs, persons who are standing may fall down.

3. Sleep paralysis

In this condition there is the inability to move the arms, legs, or entire body that occurs when a person is falling asleep or waking up. It is characterized by temporary paralysis of the body shortly before or after sleep. Sleep paralysis may be accompanied by visual, auditory or tactile hallucinations. People who experience sleep paralysis may become very anxious and often regain movement only if they hear a loud noise or another stimulus.

4. Hypnagogic hallucinations or pre-sleep dreams are dream-like hallucinations that occur in the transition between being awake and being asleep. Often, they are very vivid, frightening dreams.

2.8 Treatment and Management of Sleep Disorders

Treatment for sleep disorders depends on the cause and may include improvements in sleep hygiene (e.g., going to bed at the same time each day) and lifestyle modifications (e.g., avoiding caffeine, exercising daily, weight loss), medications, and other treatments. (NSF 2014b), (Melinda Smith et al. 2013) & (Lawrence Epstein 2014).

A. Sleep disorder can be treated on the basis of its type

1. Insomnia

If sleep studies do not indicate a pathological (related to disease) cause, improving "sleep hygiene" is the best way to treat insomnia. This means consuming less caffeine,
avoiding exercise late in the evening, and engaging in a regular relaxation routine before bedtime. For some people, watching TV at night is actually too stimulating and may keep them from falling asleep. In most cases, sedatives should only be used on a short-term basis; however, some people require long-term drug therapy. Antidepressants (e.g., trazadone [Desyrel®]) may be effective in these patients.

2. Sleep Apnea

For patients who are overweight, a weight loss program can be helpful in treating obstructive sleep apnea. Avoiding sleeping on the back also can help relieve the condition. Devices also are available that a person can wear during sleep. A CPAP (continuous positive airway pressure) machine can be used to apply pressure to the upper airway, preventing obstruction and keeping the airway open. Patients wear a small mask connected to the machine that provides pressure while they are sleeping. Patients with treatable conditions, such as enlarged tonsils or a large deviated septum, may benefit from surgery. Patients with sleep apnea should never take sleeping pills because they can prevent the person from waking up enough to start breathing again.

3. Restless Legs Syndrome & Periodic Limb Movement Disorder

These disorders are generally treated using medication.

4. Narcolepsy

There is no cure for narcolepsy, but symptoms can be managed with medication. Drugs used to treat symptoms of narcolepsy include stimulants (e.g., methylphenidate [Ritalin®], modafinil [Provigil®]), tricyclic antidepressants (e.g., imipramine, chlorimipramine), selective serotonin reuptake inhibitors (SSRIs; e.g., fluoxetine [Prozac®], and central nervous system depressants (e.g., sodium oxybate [Xyrem®]).

Many of these drugs are addictive and can have serious side effects. It is important for people with narcolepsy to get a good night’s sleep
B. Treating insomnia by identifying its cause

There are several factors to be considered when determining the cause of insomnia. First, it is helpful to know whether the insomnia is due to difficulty falling to sleep (sleep-onset insomnia) or difficulty staying asleep (sleep-maintenance insomnia). Sleep laboratory research has shown that 50% of all insomnia is due to psychological distress, especially anxiety depression.

1. **Hypoglycemia**

   Low blood sugar is a common cause of sleep-maintenance insomnia. A diet high in refined carbohydrates (breads, pasta and sugar) can lead to problems in blood sugar metabolism.

2. **Lack of exercise**

   A lack of exercise can contribute to restlessness at night. Twenty to 40 minutes of aerobic exercise on a daily basis in the morning or late afternoon can substantially improve the quality of sleep at night as well as the sense of vitality while awake.

3. **Restless legs syndrome:**

   (RLS; also known as periodic limb movement disorder or PLMD) Refer to Restless Legs Syndrome & Periodic Limb Movement for a description of this disorder. When there is no family history of this disorder, additional testing is useful to rule out a low-grade iron deficiency or folic acid deficiency. Often there can be a sub-clinical deficiency in these nutrients and supplementing them can resolve the imbalance and help support a good night's sleep. Magnesium and vitamin E are also very helpful.

4. **Other obstacles to restful sleep**

   Despite the popular lore about a nightcap before bed for a restful night, alcohol is a major contributor to sleep deprivation. Drinking even one drink 2 or 3 hours before bed can create problems. Some people are more reactive to alcohol than others, and often the only way to determine if this is a factor is to abstain for several nights and note your sleep patterns.
Side effects with sleep medications

There are various side effects associated with both over-the-counter and prescription sleep medications and it includes:

- **Drug tolerance.** Pile up of pills leads to drug tolerance which can lead to more side effects.

- **Drug dependence.** Unable to sleep or have even worse sleep without it having pills. Mostly conditions are psychological.

- **Withdrawal symptoms.** When medication are stopped abruptly, withdrawal symptoms may appear, such as nausea, sweating and shaking.

- **Side effects.** There are several side effects to sleep medications, such as drowsiness the next day, confusion, forgetfulness and dry mouth. These side effects can be severe.

- **Drug interactions.** Taking two medicines together may be harmful as sleeping medications can interact with them. This can worsen side effects and be dangerous with medications like prescription painkillers and other sedatives.

- **Rebound insomnia.** After stopping the medication, sometimes the insomnia can become even worse than before.

- **Masking an underlying problem.** There may be an underlying medical or mental disorder, or even a sleep disorder, that if treated would provide more relief from insomnia.

2.9 Natural Therapies for Sleep Disorders

When sleep disorder is due to nervousness, anxiety or depression, temporary measures can encourage sleep during a crisis so that the immune system does not get too run down. The following botanical treatments, homeopathic combinations and nutrient supplements can be supportive for short periods of time.
Botanical treatments

- Valarian (*Valeriana officinalis*): there are many reports on use of *Valeriana officinalis* for the treatment of sleep disorder (Barton et al. n.d.).

- Passionflower (*Passiflora incarnata*): It is used to relieve stress, anxiety and help with sleep and relaxation (Ray Sahelian 2014).

- Skullcap (*Scutellaria laterifolia*): used for anxiolytic agents; sleep disorders; anticonvulsants; muscle spasms (Wolfson & Hoffmann 2006).

- Kava (*Piper methysticum*): very effective in treating sleep disorder (Hechtman 2012).

- Chamomile: Tea of chamomile is effective for sleep disorder (Srivastava et al. 2010).

- Peppermint: Oil of peppermint helps in soothing and sleeping activity (Webmd n.d.).

- Hops: Findings indicate that a valerian-hops combination and diphenhydramine might be useful adjuncts in the treatment of mild insomnia (Morin et al. 2005).

- lemon balm: Valerian/lemon balm may assist in reducing symptoms of sleep disorder during the menopause (Taavoni et al. 2013).

- Lavender: Reduces stress and can be used for treatment of sleep disorder (Chioca et al. 2013).

- St. John’s Wort: It enhances the level of GABA and induces sleep (Butterweck 2003).

- Natural Melatonin.

Homeopathic combinations

There are several over-the-counter formulas that can be very helpful toward getting a restful night's sleep, including:
Review of Literature

- *Calms Forte* by Hyland Homeopathics: use as directed
- *Calm Formula* by Boericke
- *Insomnia Formula* by Hylands Homeopathics: take as directed

2.10 Neurotransmitter and Sleep (Mendelson 2001)

Medications for insomnia and hypersomnia usually act on neural systems and affect, in some manner, neurotransmitters. Sleep aids tend to work on the different receptors and neurotransmitters in the brain. Neurotransmitters are the endogenous chemicals that transfers signals across synapse from one neuron to target neuron.

Any medications such as mood stabilizers, antidepressants and antipsychotics all affect this process to bring about changes. When a message comes in at one end of a nerve cell, an electrical impulse travels down the "tail" of the cell, called the axon, and causes the release of the appropriate neurotransmitter. Molecules of the neurotransmitter are sent into the tiny space between nerve cells, called the synaptic cleft (Figure 6).

![Figure 6: Neurotransmittion at synapse](image_url)
Neurotransmitters are responsible for spinal reflexes and sleep regulation. Following are neurotransmitters of interest that are involved in sleep.

a) GABA (Gamma Aminobutyric acid)

GABA is an amino acid derivative that acts as an inhibitory neurotransmitter, preventing or reducing certain nerve signals. It controls nervous signals in the retina and the central nervous system. Drugs can temporarily increase GABA levels and in turn reduce anxiety, and offer anti-convulsive effects.

The predominant effect of GABA is the interaction with a specific receptor protein which results in an increase of the chloride ion conductance of the post-synaptic membrane to produce an inhibition of neuronal firing. GABA is not found in any significant amounts outside the brain. Indeed, GABA is pretty much unique to the central nervous system of mammals. Many sedatives and hypnotics are GABA agonists. It is essential to maintain the overall balance between neuronal excitation and inhibition that is vital to normal brain function. Either imbalance in the extreme can result in death (Johnston 2005).

GABA produces neuronal inhibition by acting on an amazing diversity of membrane-bound receptors. These receptors can be divided into two major types:

1. Ionotropic receptors that are ligand-gated ion channels (GABAA and GABAC receptors), and

2. Metabotropic receptors that are G-protein coupled receptors (GABAB receptors) that act via second messengers.

Mechanisms for enhancing GABA activity

There are at least five known mechanisms by which drugs can increase the availability and activity of GABA (VCU 2002).

1. Stimulation of GABA-A receptors.

   GABA-A receptors are coupled to chloride ion channels; activation of the GABA-A receptor causes the opening of ion channels to allow the flow of negatively charged...
chloride ions into the cell or positively charged potassium ions out of the cell which further leads to negative change in the transmembrane potential, usually causing hyperpolarization. (Graham et al. 1996)

2. **Increasing the release of GABA from glial cells.**
   This is believed to be the mode of action of gabapentin, which is structurally similar to GABA but does not interact with GABA-A receptors.

3. **Inhibition of GABA transaminase.**
   GABA transaminase is the enzyme that breakdown GABA). Vigabatrin, valproate, valerenic acid are the compounds which inhibit catabolidm of GABA.

4. **Increases in GABA synthesis and release**
   This is also one of the multiple GABAergic mechanisms of valproate.

5. **Inhibition of reuptake of GABA**
   Tiagabine prevents GABA reuptake by neurons and glial cells by inhibiting the action of GAT-1 GABA transporters.

It is synthesized by a specific enzyme, l-glutamic acid decarboxylase (GAD), in one step from l-glutamate. Thus, in addition to its role in protein synthesis, in cofactors such as folic acid and in hormones such as thyrotropin-releasing hormone, and its action as a neurotransmitter itself, glutamate must be available in certain nerve endings for biosynthesis of GABA.

**b) L- Glutamic acid**

L- Glutamic acid is also known as glutamate, is an amino acid and is the most common neurotransmitter in the body. 80% of the brain's neurons release glutamate. Glutamate’s most vital function as a neurotransmitter is in cognitive activities like memory and learning. Scientists have pointed the finger at glutamic acid as being involved in epileptic seizures, probably since glutamate can also be a precursor for the synthesis of GABA. Endogenous glutamate and GABA in the PnO contribute to the regulation of sleep duration. (Watson et al. 2011)
Glutamate is involved in arousal and anesthetic drugs seem to work at least partly by reducing neurotransmission normally regulated by glutamate. Lower than normal levels of oxygen in the blood such as apnea cause stimulate production of glutamate.

c) Acetylcholine
Ach was the first neurotransmitter to be discovered by scientists. Ach have very much important in the sleep process. Ach have cell bodies in two area of brain the Pons and the Basal Forebrain. It is involved in the scheduling of REM sleep. The activity of ACh neurons, in general, is associated with cortical arousal (increase in wave frequency) and desynchrony, as measured by an EEG. The onset of Alzheimer’s disease happens when some regions of the brain have depleted acetylcholine. The preoptic area and anterior hypothalamus (together sometimes called POAH) are important in both REM and in regulation of the body's temperature during sleep. It is also to make the causal direction go the other way by manipulating the POAH it is possible to include insomnia or sleepiness, and if the POAH is artificially warmed, the brain is induced to go into deep sleep. The temperature of both the brain and the body falls during NREM sleep. The longer the NREM-sleep episode, the more the temperature falls. By contrast, brain temperature increases during REM sleep. POAH plays a combined role indicate that: a) lesions of the basal forebrain create insomnia, while stimulation creates drowsiness and sleepiness; b) Warming the POAH induces delta sleep; and c) POAH nerve firing increases during sleep and in response to an increase in body temperature (Vazquez & Baghdoyan 2001).

d) Norepinephrine
This neurotransmitter most involved in the “fight or flight” response and other stressful situations, since it increases heart rate and blood pressure. Studies have shown that elevated norepinephrine levels are implicated in symptoms in some mood disorders. When these neurons in the locus coeruleus in the bottom of the brain are stimulated, the cortical area of the brain becomes more active. Norepinephrine is therefore thought to be instrumental in causing people to wake up. The NE-deficient mice showed a significantly shorter latency to sleep under many different conditions, measured both behaviorally and
with electroencephalography. These data suggest that NE is wake promoting during the period of time between a mildly stressful event or a low dose of amphetamine and sleep onset. The Norepinephrine-deficient mice did not show deficits in wake or increases in rapid eye movement sleep, as predicted from current models of the involvement of Norepinephrine in the regulation of these 2 states (Hunsley & Palmiter 2003). Norepinephrine promotes wakefulness during transitions between sleep and wake under conditions involving mild stress and SD, but not under baseline circumstances (Hunsley & Palmiter 2004).

e) **Dopamine**  
Another inhibitory neurotransmitter involved in voluntary movement and motivation. Sometimes called the "salience chemical" dopamine plays important roles in pleasure and subjective feelings of happiness. Alcohol, nicotine and some recreational drugs increase the level of dopamine. Schizophrenia is linked to elevated levels of dopamine in the frontal lobes of the brain. These results are interesting as they demonstrate a mechanism in which dopamine, normally increased at times of stimulation, can directly inhibit production and release of a molecule, melatonin, that induces drowsiness and prepares the body for sleep (Anon 2012).

f) **Serotonin**  
Serotonin is involved in several important body functions such as memory, emotions, moods, appetite and thermoregulation. It is important in regulating sleep-waking. Serotonin deficiencies have been linked to depression, anger, OCD, sleep disturbances, irritable bowel syndrome and many other emotional and physical disturbances. Serotonergic helps in modulation on the sleep/wake cycle takes place through a multitude of post-synaptic receptors which mediate different or even opposite responses (Portas et al. 2000).

g) **Steroids**  
Steroids also participate in sleep regulation. Cortisol increases propensity to REM sleep and estrogen supplements appear to help post-menopausal women sleep, although this
may be due to an indirect mechanism. Growth hormone-releasing hormone (GHRH) is a common regulator of the sleep EEG and nocturnal hormone secretion. In healthy volunteers GHRH prompts an increase in the amount of slow wave sleep (SWS) and in growth hormone (GH) secretion and blunting of cortisol release. Inhibition of GHRH may contribute to sleep-endocrine aberrances during depression (Steiger et al. n.d.) and corticotropin-releasing hormone also play parts in sleep regulation (Schüssler et al. 2006).

h) Orexin
Orexin (Hypocretin) system as a critical regulator of sleep/wake states as well as feeding behavior and reward processes. Orexin deficiency results in narcolepsy in humans, dogs, and rodents, suggesting that the orexin system is particularly important for maintenance of wakefulness. In addition, orexin deficiency also cause abnormalities in energy homeostasis and reward systems. Orexin activates waking active monoaminergic and cholinergic neurons in the hypothalamus and brainstem regions to maintain a long, consolidated waking period (Tsujino & Sakurai 2009)

i) Adenosine
Adenosine is neither stored nor released as a classical neurotransmitter and is thought to be formed inside cells or on their surface, mostly by breakdown of adenine nucleotides. It is produced during energy metabolism and plays a part in the sleep homeostatic process as it inhibits wake-promoting neurons. These neurons are in the basal forebrain, and damage or changes to these neurons seems to play a part in the development of Alzheimer’s disease. The extracellular concentration of adenosine increases in the cortex and basal forebrain during prolonged wakefulness and decreases during the sleep recovery period. Therefore, adenosine is proposed to act as a homeostatic regulator of sleep and to be a link between the humoral and neural mechanisms of sleep-wake regulation. Both the adenosine A (1) receptor (A (1) R) and A (2A)R are involved in sleep induction. The A(2A)R plays a predominant role in the somnogenic effects of PGD(2) (Huang et al. 2011). Caffeine seems to work by affectinv adenosine.
2.11 GABA-A receptor

GABA-A receptor are the ligand gated ion channel due to ions channel there is fast neurotransmission (Stephenson 1988). The GABA-A receptor consists of pentameric transmembrane proteins and a central Cl⁻ channel pore which allows the influx of chloride ions inside membrane and cause hyperpolarization of membrane (Chebib & Johnston 2000). Most GABA-A receptors are composed of two α subunits, two β subunits and one γ subunit. There are 18 different sub- subunits comprising the GABA-A receptor family: α1–6, β1–3, γ1–3, δ, ε1–3, θ, and π which forms diverse compositions and their corresponding functions (Jacob et al. 2008). GABA-A receptors mediate a wide range of pharmacological effects including sedation, anxiolysis, and muscle relaxation due to the diversity of their subunit composition. The sedative-hypnotic effects of pharmacological compounds are mediated by GABAA receptors that contain the α1 subunit (Mohler 2006). Various drugs like zolpidem, zaleplon, zolpidem and indiplon available in market to treat sleep disorders shows some selectivity for α1 subunit containing GABA-A receptors, acting as positive allosteric modulators (Johnston 2005). The sedative effects of the α1-selective agent zolpidem are diminished in the α1 knock-in mouse, consistent with sedation being mediated via α 1-containing GABA-A receptors (Crestani et al. 2000).

The α1 subunit constitutes 60% of the GABAA receptor population and is widely distributed in the target areas of sleep-promoting pathways, e.g., cerebral cortex, thalamus, and hypothalamus. Non-benzodiazepine hypnotics bind with higher relative affinity to alpha1-containing receptors (Ebert et al. 2006).

Various herbals are reported to have ability to activate GABA receptor like chamomile and green tea, red wine, absinthe, Gingko and Valerian. The significant actions on recombinant GABA receptors is due to various components present I them like flavonoids and terpinoids, the activation of GABA receptors have pharmacological effects of treatment of insomnia (Lecture & Society 2003).
2.12 GABA shunt Pathway

GABA and L-glutamate are the most abundant neurotransmitters in mammalian brain. GABA is the major inhibitory neurotransmitter and L-glutamate is an excitatory neurotransmitter. Balance between these two is essential for normal brain function. The GABA shunt is a closed-loop process and pathway. It serves function of synthesis of GABA, metabolism and conserving the supply of GABA.
The first step in the GABA shunt is the transamination of α-ketoglutarate, formed from glucose metabolism in the Krebs cycle by GABA α-oxoglutarate transaminase (GABA-T) into l-glutamic acid. Glutamic acid decarboxylase (GAD) catalyzes the decarboxylation of glutamic acid to form GABA. Furthermore, expression of GAD and some GABA receptor subunits have been demonstrated in some non-neural tissues, indicating the likely function of GABA outside of the CNS. GABA is metabolized by GABA-T to form succinic semialdehyde. To conserve the available supply of GABA, this transamination generally occurs when the initial parent compound, α-ketoglutarate, is present to accept the amino group removed from GABA, reforming glutamic acid. Therefore, a molecule of GABA can be metabolized only if a molecule of precursor is formed. Succinic semialdehyde can be oxidized by succinic semialdehyde dehydrogenase (SSADH) into succinic acid and can then reenter the Krebs cycle, completing the loop. GABA in glia is converted to glutamine, which is transferred back to the neuron, where glutamine is converted by glutaminase to glutamate, which re-enters the GABA shunt (Olsen & DeLorey 1999).

![GABA shunt Pathway](image)

**Figure 10: GABA shunt Pathway**

### 2.13 GC-MS an analytical tool to determine phyto-constituents

Valerian is the common name given to the crude drug consisting of the underground parts of species of Valeriana. Valerians are known to be reservoir of mixture of essential oils.
Complex mixture are mostly comprises of rich volatile components like lipids, terpenoids, ketones, phenols, oxygenated derivatives like antimicrobial, insecticidal and antioxidants (Burt 2004).

*Valeriana jatamansi* was used as traditional medicine for the treatment of anxiety disorders (H. Z. Zheng 2012) and now also anxiolytic property have been reported and used in clinical prescription (Yan et al. 2011). According to some reports *Valeriana jatamansi* contains iridoids, and flavonoids as components of essential oils (Lin et al. 2010) & (Bhatt et al. 2012), but the active compounds of *Valeriana jatamansi* which target directly for sleep disorder mainly insomnia have not been adequately elucidated. Some report states that valtrate at a high dose gives sedative properties by inhibiting spontaneous motion and increasing the sleeping number induced by pentobarbital sodium in mice (Chen et al. 2003). But the proper mechanism has not been reported.

Valerian continues to be a safe sedative/hypnotic choice for patients with mild to moderate insomnia (Hadley & Petry 2003). GC-MS screening of the essential oil of the *Valeriana wallichii* mostly consists of sesquiterpenes, terpenes like maaliol, santalene, acoradiene, β-gurjune and guaiol which together may be responsible for antinociceptive analgesic effect (Sah et al. 2010) & (S.P. Sah 2012).

### 2.14 HPTLC Method Development for Determination of GABA

Thin layer chromatography (TLC), also known as planar-chromatography or flat bed chromatography is like all other chromatographic techniques, a multistage distribution process. HPTLC is the most simple separation technique today available to the analyst. It can be considered a time machine that can speed your work and allows you to do many things at a time usually not possible with other analytical techniques.

Various analytical techniques such as fluorimetry (Lindgren et al. 1982), high performance liquid chromatography (HPLC), followed by electrochemical (Khuhawar & Rajper 2003) & (Kono & Himeno 2000) or spectrofluorimetric detection (Griesmann et al. 1982), gas chromatography (GC) (Pearson & Sharman 1975) and capillary electrophoresis
(Baldacci et al. 2003) are employed to estimate GABA in biological samples. Since most of the amino acids are aliphatic in nature, they require derivatization in order to be detected by ultraviolet (UV)–visible detectors. In liquid chromatography methods like HPLC and GC, complex procedures like precolumn derivatization with various ion pairing reagents and electrochemical detection are applied for the separation and quantification of GABA (Kehr & Ungerstedt 1988). Further, the experimental procedure and complexity in the sample preparation for the above-mentioned techniques are tedious. In view of the biological importance, there has been a spurt of interest in the development of new analytical methods to estimate GABA in brain tissue (Saravana Babu et al. 2011).

Factors influencing the TLC / HPTLC separation and resolution of spots:

- Type of stationary phase (sorbent),
- Type of pre-coated plates (TLC / HPTLC); for quantitative analysis, use of HPTLC pre-coated is absolutely essential,
- Layer thickness / Binder in the layer,
- Mobile phase(solvent system),
- Solvent purity,
- Size of the developing chamber,
- Saturation of chamber (pre-equilibrium),
- Sample volume to be spotted,
- Size(diameter) of the initial spot,
- Solvent level in the chamber,
- Gradient,
- Relative humidity,
- Temperature (R_f values usually increase with rise in temperature),
- Flow rate of solvent,
- Separation distance,
- Mode of development.
Features of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard.
2. Several analysts work simultaneously.
3. Lower analysis time and less cost per analysis.
4. Low maintenance cost.
5. Simple sample preparation - handle samples of divergent nature.
6. No prior treatment for solvents like filtration and degassing.
7. Low mobile phase consumption per sample.
8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination.
10. Non UV absorbing compounds detected by post-chromatographic derivatization.

Steps involved in HPTLC:

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning
9. Documentation of chromatic plate

HPTLC offers many advantages over other chromatographic techniques such as unsurpassed flexibility (esp. stationary and mobile phase), choice of detection, user friendly, rapid and cost effective. Thus, HPTLC is most widely used at industrial level for routine analysis of herbal medicines.