Chapter 1

Introduction and Review of Literature
Introduction

Anxiety is the apprehension of danger or something unpleasant (Barchas and Altemus, 1999). Anxiety can be conceived as an emotional anticipation of an aversive situation, which is likely to occur and difficult to predict and control (Landgraf, 2001). It is a central nervous system disorder (Kjernisted and Bleau, 2004; Weinberger, 2001), a common emotional phenomenon in humans (Clement et al., 2002) which is emerging to be a modern day liability. Stress and anxiety are common psychiatric manifestations of the modern world and lifestyles. In minuscule quantities, stress and anxiety are good as they can motivate and help one be more productive. However, if it becomes excessive it becomes problematic as it interferes in our daily activities and messes-up life. Persistent and unrelenting stress often leads to anxiety and unhealthy behaviours. Anxiety is the most common mental illness affecting one eighth of the total population and has become a very important area of research in psychopharmacology in the current decade (Jung et al., 2006).

Anxiety in society

Anxiety disorders are more prevalent than any other form of mental illness (DuPont et al., 1996). Anxiety Disorders are known to affect about 40 million American adults aged 18 years and older (about 18 %) in a given year (Kessler et al., 2005) causing them to be filled with fearfulness and uncertainty (US Department of Health and Human Services, 2009). Of this only 4 million will receive treatment, and of those, only 400,000 will receive proper treatment (Folk and Folk, 2015).

Anxiety disorders are psychiatric disorders affecting nearly 25 % of the adult population at some point in their life (Fig. 1.1). The prevalence of anxiety disorders is 30.5 % and 19.2 % in women and men respectively. The prevalence of anxiety disorders is surprisingly high in young populace. Children aged 7 to 11 years reported a 15.4 % prevalence rate of anxiety disorders. A survey also stated that less than 14 % of people with such psychiatric disorders receive treatment (Leon et al., 1995). Anxiety can aggravate many physical and mental ailments and also impede recovery from any other problems.

The impact of anxiety disorders on the economy is huge. The overall cost of mental illness in American populace in 1990 was $ 147.8 billion and the costs associated with anxiety disorders were $ 46.6 billion, accounting for 31.5 % of the
total. Morbidity costs for anxiety disorders were $34.2 billion in 1990. Mortality costs of $1.3 billion represented only 2.7% of all anxiety disorder costs. Most of these costs were related to suicides, 75% of which were among males (DuPont et al., 1996).

Anxiety is one of the most common psychological disorders in school-aged children and adolescents worldwide (Costello et al., 2003). Anxiety in children is associated with poor social and coping skills, often leading to avoidance of social interactions (Albano et al., 2003; Weeks et al., 2009), loneliness, low self-esteem, perceptions of social rejection, and difficulty forming friendships (Bokhorst et al., 2001; Weeks et al., 2009).

**Fig. 1.1**

![Global prevalence data for common mental disorders](image)

**Fig. 1.1: Global prevalence data for common mental disorders.** Data is averaged across major depressive disorders, dysthymia and anxiety disorders. Taken from Baxter et al., 2013.

**Prevalence of anxiety disorders in India**

Anxiety measured in adolescents in Kolkata, India using a self-report semi-structured questionnaire and State-Trait Anxiety Inventory (STAI) showed that 20.1% of boys and 17.9% of girls were found to be suffering from high anxiety. More boys were anxious than girls ($p < 0.01$). A group of 460 adolescents (220 boys and
240 girls, aged 13-17 years were recruited to participate in this study via a multi-stage sampling technique (Deb, et al., 2010).

Another survey of the Indian scenario on psychiatric disorders employing 33,572 subjects revealed that 4.2 % of the population suffered from phobia, 5.8 % from generalized anxiety disorder, 3.1 % from obsession and 4.5 % from hysteria. This meta-analysis study also revealed that all neurotic disorders were significantly high among females (32.2 % vs. 9.7 %, $p < 0.01$) (Reddy and Chandrashekar, 1998).

Another study by Ganguli, (2000) reported a prevalence rate of 16.5 % for anxiety disorders with rural to urban ratio of 100:106. Madhav, (2001) reported 18.5 per 1000 prevalence rate for anxiety disorders. Most of these studies did not assess the prevalence rate for individual anxiety disorders except for the study by Reddy and Chandrashekar (1998).

**Etiology of anxiety**

The etiology of anxiety disorders is becoming an area of major interest and importance owing to high prevalence of the disorders and the accompanying impairment (Leyfer et al., 2009). In addition to causing human suffering, anxiety disorders entail significant economic burden (Rice and Miller, 1998). The etiology of anxiety disorders has been studied through a number of approaches including genetic and family studies to study the familial aggregation of the disorders (Woodman, 1993). Many anxiety disorders start out in childhood (Keller et al., 1992; Ost, 1987; Stemberger et al., 1995) and may continue into adulthood if left untreated (Cantwell and Baker, 1989; Dadds et al., 1997). Research suggests anxiety to aggregate in families (Last et al., 1991; Weissman et al., 1984). Several empirical studies stress on the importance of upbringing of children, familial aggregation on anxiety. A review article by Ballash et al., (2006) exhaustively studies the role of family and anxiety and also provides recommendations for future research. Several studies have reported that children aged 7-17 years were three times at a higher risk of developing anxiety disorders if any of the parents had an anxiety disorder or a substance abuse disorder or even if neither parent ever had (Dierker et al., 1999; Merikangas et al., 1998 and 1999). In another study (Turner et al., 1987), children of parents with anxiety disorders were seven times more prone to develop an anxiety disorder when compared to children of parents without any psychiatric diagnosis. According to Vasey and Dadds, (2001) a dynamic interplay of various potential predisposing factors play
critical roles in the pathogenesis of anxiety. Predisposing factors include insecure attachment between parents and their offspring’s further enhancing children’s vulnerability to anxiety (Calkins and Fox, 1992). Several studies have also reported that behavioural inhibition due to insecure attachment between parents and their offspring’s significantly contributes to the development of anxiety disorders (Kagan et al., 1988; van Brakel et al., 2006; Biederman et al., 1990).

Multiple molecular genetics approach has been applied to identify several candidate chromosomal regions, genes, and polymorphisms for anxiety disorders. The results are conflicting and no specific genes have been identified as such, instead they categorised these disorders complex, caused by an interaction among a variety of genes as well as environmental factors (Smoller et al., 2000). Leyfer et al., (2006), showed a causal relationship between anxiety and deletion of chromosome 7q11.23 (Karayiorgou and Gogos, 1997). However further studies are warranted to identify how deletion of 7q11.23 may contribute to anxiety (Leyfer et al., 2009).

**Anxiety disorders**

Anxiety and fear are normal emotional responses to threatening situations. In human anxiety disorders such as panic disorder, obsessive–compulsive disorder, post-traumatic stress disorder, social phobia, specific phobias and generalized anxiety disorder, these responses are exaggerated (Hovatta et al., 2005). Each anxiety disorder has myriad symptoms and all these symptoms cluster around excessive, irrational fear and dread (US Department of Health and Human Services, 2009).

Classically, in its non-pathological form anxiety is distinguished into the ‘state’ and the ‘trait’ anxiety. “State anxiety” is an anxiety that a subject experiences at a particular moment and is increased by the presence of an anxiogenic stimulus. In contrast, “trait anxiety” does not vary from moment to moment and is considered to be an “enduring” feature of an individual or long-term tendency of an individual to show an increased anxiety response (Beuzen and Belzung, 1995; Lister, 1990; Spielberger et al., 1970; Leonardo and Hen, 2006).

The standard reference for categorization of mental disorders is the *Diagnostic and Statistical Manual* (DSM-IV) of the American Psychiatric Association (Barchas and Altemus, 1999). According to the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) (First, 1994), anxiety is categorised into the following disorders:
Chapter 1 - Introduction and Review of Literature

Panic attack

During a panic attack there is sudden onset of intense apprehension, fearfulness or terror often associated with the feeling of impending doom. A panic attack is accompanied by physical symptoms such as shortness of breath, palpitations, chest pain or discomfort, dry throat and feeling of having a heart attack or stroke. Persons experiencing a panic attack report an urgent desire to run-away or flee from wherever the attack is occurring.

Panic disorder

Panic disorders are characterised by the presence of recurrent, unexpected panic attacks accompanied by persistent concern and worry about having another panic attack and the possible implications or consequences. Panic disorder is often accompanied by other problems such as depression, drug abuse or alcoholism. Panic disorders are treatable responding to medications or certain kinds of cognitive psychotherapy. Panic disorder is known to affect about 6 million American adults (Kessler et al., 2005) and often begins in late adolescence or early adulthood (Robins and Regier, 1991) and are inherited (The NIMH Genetics Workgroup). Fig. 1.2 describes the brain regions implicated in panic disorder.

Fig. 1.2

![Image](image-url)

**Fig. 1.2: Panic disorder: Brain regions implicated** - Excessive neurotransmitter activity between cortex, thalamus, hippocampus, amygdala, hypothalamus and periaqueductal grey matter has been implicated in panic disorder. Increased serotonin activity in the amygdala and frontal cortex induces symptoms of anxiety (defensive behaviours and postural freezing) and hyperactivity in the periaqueductal grey. The locus coeruleus facilitates increased noradrenaline release mediating physiological and behavioural sympathetic nervous system (CNSforum, 2000)
Agoraphobia

Agoraphobia is anxiety experienced in places or situations from which escape might be difficult or a forethought that help may be not available if having a panic attack or panic-like symptoms occur. Agoraphobia brings in marked decrement in the individual’s performance in tasks performed outside home or in crowd or while travelling.

Specific phobia

Specific phobia is anxiety experienced on confronting the phobic stimulus. It is clinically significant anxiety provoked by exposure to a specific feared object or situation often leading to avoidance behaviour. Sometimes a full-blown panic attack is experienced in response to the phobic stimulus when the person realises that he has to remain in the same situation or believes that escape is impossible. The incidence of specific phobias is greater in females

Social phobia (Social Anxiety Disorder)

Social phobia is persistent fear of social or performance situations as the person anticipates embarrassment. Common associated features of social phobia include low self-esteem, feelings of inferiority, sensitive to criticism, negative evaluation etc. Epidemiological and community based studies have suggested it to be more common in women but clinical studies contradict saying an equal representation of both the sexes or is more common among males. Fig. 1.3 summarizes the neural network involved in phobia.
Chapter 1 - Introduction and Review of Literature

**Fig. 1.3**

**Fig. 1.3: Phobia: Brain regions implicated** - Activation of amygdala causes anticipatory anxiety or avoidance while activation of the hypothalamus activates the sympathetic nervous system. Other brain areas implicated include the thalamus and cortical structures which form a key neural network along with amygdala. Noradrenaline release stimulated by the locus coeruleus mediates physiological and behavioural arousal (CNSforum, 2000).

**Obsessive compulsive disorder (OCD)**

OCD is anxiety characterized by obsessions, usually worries unrelated to real-life problems and compulsions or repetitive behaviours that a person feels driven to perform in response to an obsession to reduce anxiety or distress. Obsessive compulsive disorder affects about 2.2 million American adults (Kessler et al., 2005) and has familial aggregation and one-third of adults develop symptoms in childhood. Fig. 1.4 depicts regions of the brain implicated in OCD.
Fig. 1.4: OCD: Brain regions implicated - Increased metabolic activity in the cortico-basal ganglia network is seen in patients with OCD. Hyperactivity in the neurotransmitter circuits between cortex, basal ganglia and thalamus has been implicated in OCD. Decreased activity of the serotonergic neurons arising from rostral raphe nucleus may result in lack of inhibitory effect in OCD. Further, excessive activity of dopaminergic neurons arising from the substantia nigra may produce excessive excitation in the brain areas implicated in OCD (CNSforum, 2000).

Post-traumatic stress disorder (PTSD)

PTSD occurs following exposure to an extreme traumatic event. Traumatic events could include violent personal assault (sexual assault, physical attack or torture, natural or manmade disaster, an accident or as a prisoner of war or being diagnosed with a life threatening illness or a witness to any one of these events). Persons with PTSD have recurrent and intrusive recollections of the traumatic event or recurrent distressing dreams. Persons experiencing such symptoms for more than a month are specified as having PTSD. PTSD affects about 7.7 million American adults (Kessler et al., 2005). Women are more vulnerable than men (Davidson, 1999), and also has familial aggregation (Yehuda, 1999). Fig. 1.5 depicts the neural network implied in PTSD.
Fig. 1.5: **PTSD: Brain areas implicated** - The regions of the brain involved in memory processing that are implicated in PTSD include hippocampus, amygdala and frontal cortex. The heightened stress response involves the thalamus, hypothalamus and locus ceruleus (CNSforum, 2000).

**Acute stress disorder**

It has symptoms similar to PTSD but develops within one month after exposure to an extreme traumatic stressor.

**Generalised anxiety disorder (GAD)**

GAD is excessive anxiety and worry lasting for about 6 months. The anxiety and worry are accompanied by any of the following symptoms viz. restlessness and fatigue, difficulty in concentrating, irritability, muscle tension and disturbed sleep. Those suffering from these disorders have excessive worry about routine life matters their job, finances, house-hold chores, health of their family members etc. It is usually accompanied by somatic symptoms like cold hands, dry mouth, urgent desire to urinate or diarrhoea, nausea, trouble swallowing etc. The disorder is more prevalent among women than men. It affects 6.8 million American adults (Kessler et al., 2005) with twice as many women as men being affected (US Department of Health and Human Services, 2009). Fig. 1.6 shows the brain areas implicated in GAD.
Fig. 1.6: GAD: Brain regions implicated - Patients with GAD experience hyperactive neurotransmitter circuits between the cortex, thalamus, amygdala and hypothalamus. Hypofunction of the serotonergic neurons arising from dorsal raphe nucleus and GABAergic neurons that are extensively distributed in the brain may result in a lack of inhibitory effect on the putative GAD pathway. Moreover, overactivity of the noradrenergic neurons arising from the locus coeruleus may produce excessive excitation in the brain areas implicated in GAD (CNSforum, 2000).

Physical symptoms during an anxiety attack

As discussed, each anxiety disorder is characterised by its own physical symptoms. The general symptoms experienced during any anxiety attack include a feeling of impending doom, foreboding, rapid heartbeat and breathing, sweating, twitching and trembling, muscle tension, dry mouth and difficulty in swallowing, depersonalisation, blurred vision, dizziness, diarrhoea or immediate need to urinate, nausea, plugged ears, irritability and loss of temper, freaking out etc. All these symptoms cluster irrational fear and can vary from person to person in their intensity and frequency. However, they are part of a system that is designed to keep one safe and do not cause any harm. They can be harmful only when they occur in situations when one is not physically threatened thereby disrupting his routine or normal life (Folk and Folk, 2015).
Chapter 1 - Introduction and Review of Literature

Biochemistry of anxiety

Anxiety is recognized as one of the most important emotional processes with firm neurobiological roots. Most information has come from studying the action of anxiety-reducing, or anxiolytic drugs. The introduction of benzodiazepines has also set the stage for greatly increasing our understanding of the biochemistry of anxiety (Barchas and Altemus, 1999). The use of benzodiazepines into clinical practice has increased enormously owing to their efficacy, safety and tolerability. However, their mechanism of action was unknown until 1977, when it was discovered that benzodiazepines interacted with specific receptors in the central nervous system (Haefely, 1978). In 1987, the GABA<sub>A</sub> (gamma-Aminobutyric acid) - benzodiazepine receptor was isolated and sequenced (Schofield <i>et al.</i>, 1987) and was visualised by electron microscopy in 1994 (Nayeem <i>et al.</i>, 1994).

Fig. 1.7

A.  

B.  

Fig. 1.7: GABA<sub>A</sub> receptor - (A). A heteropentameric glycoprotein with five protein subunits arranged around a central pore. The most abundant GABA<sub>A</sub> receptor subunits arrangement in the brain is α<sub>1</sub>β<sub>2</sub>γ<sub>1</sub>. (B). Each subunit has a large extracellular N-terminal domain comprising part of the agonist/antagonist binding site followed by three membrane spanning domains and a fourth membrane spanning domain with C-terminal end being extracellular (CNSforum, 2000).

The GABA<sub>A</sub>-benzodiazepine receptor complex has five protein subunits and is permeable to chloride and other ions (Fig. 1.7 A and B) (Nutt and Malizia, 2001). The benzodiazepines interact with the GABA<sub>A</sub> subtype of GABA receptor, primarily postsynaptically, and mediate changes in neuronal membrane potential by opening Cl<sup>-</sup> channels. GABA and the benzodiazepines each allosterically modulate the
binding of the other to this macromolecular complex: the benzodiazepines act by binding to α subunit and GABA by binding to the β subunit. Benzodiazepines facilitate GABAergic transmission primarily by increasing the frequency of Cl⁻ channel opening in response to binding of the GABA<sub>A</sub> receptor by GABA (Barchas and Altemus, 1999). Thus, benzodiazepine ligands do not directly open the channel but instead enable GABA to open the channel resulting in augmentation or diminution of its inhibitory effects (Fig. 1.8) (Barnard <i>et al.</i>, 1998). Benzodiazepine binding allosterically changes the receptor complex to increase the efficiency of GABA thereby producing a larger inhibitory effect. Benzodiazepines differ from barbiturates, chloral hydrate, chlormethiazole and ethanol which in addition to enhancing GABA can also directly open the chloride channel. Owing to their direct action on the chloride channel, these drugs can be fatal when overdosed.

**Fig. 1.8**

![GABA<sub>A</sub>-benzodiazepine receptor complex](image)

**Fig. 1.8: GABA<sub>A</sub>-benzodiazepine receptor complex** - Benzodiazepines bind to the gamma subunit of the GABA<sub>A</sub> receptor. They act as positive allosteric modulators of the GABA<sub>A</sub> receptor and binding of GABA to the alpha subunit is enhanced by benzodiazepine, resulting in greater influx of Cl⁻ ions (CNSforum, 2009).

One of the most widely accepted mediators known to play a central role in the pathophysiology of anxiety disorders is the gamma aminobutyric acid (GABA) system (Lydiard, 2002). Benzodiazepenes, neuroactive steroids, and barbiturates act
as allosteric modulators of the GABA<sub>A</sub> receptor, b-carboline and barbiturates function as direct GABA agonists. Valproate, gabapentin, pregabalin, and vigabatrin increase brain GABA levels or neurotransmission by targeting the metabolic pathways of GABA. Tiagabine selectively increases synaptic GABA availability by blocking the reuptake of GABA via transporter inhibition. These agents have been shown to possess anxiolytic properties, owing largely to their mechanisms of action (Nemeroff, 2003).

Brain imaging and functional studies have shown that the neurobiology of anxiety is interplay of several neurotransmitters (Cates et al., 1996; Sandford et al., 2000; Millan, 2003; Augustin, 2005).

**GABA** (gamma-Aminobutyric acid) is the most important inhibitory transmitter of the central nervous system and is widely distributed throughout the central nervous system (Nutt and Malizia, 2001). GABA works to regulate the neuronal excitability and thereby serves as a ‘brake’ on the neuronal circuitry during stress and is the brain’s natural stress reliever (Weeks, 2009). An increase in its activity favours sedation, amnesia and ataxia, whereas, a mildest attenuation results in arousal, anxiety, restlessness, insomnia and exaggerated activity. Binding of GABA to GABA<sub>A</sub>-benzodiazepine receptor complex causes a conformational change in the receptor, thereby increasing the permeability to chloride ions. This chloride influx hyperpolarises the neuron, reducing its excitability and bringing about an inhibitory effect on neuronal activity (Nutt and Malizia, 2001).

The major metabolic pathway of GABA is from \( \alpha \)-ketoglutarate synthesised from TCA (tricarboxylic acid) cycle to succinate via glutamate, GABA and succinic semialdehyde. It is called the GABA shunt as it bypasses the TCA cycle. Firstly, glutamate is produced from \( \alpha \)-ketoglutarate and is converted to GABA by a decarboxylation reaction catalysed by glutamate decarboxylase (GAD). GAD is the rate limiting enzyme in GABA synthesis and it requires pyridoxal phosphate (PLP) as its cofactor (Roberts and Kuriyama, 1968). This conversion of glutamate to GABA is essentially reversible. GABA catabolism is catalysed by GABA transaminase (GABA-T) which produces succinic semialdehyde (SSA) from GABA with stoichiometric conversion of \( \alpha \)-ketoglutarate to glutamate. The second step of GABA catabolism is catalysed by rapid oxidation of SSA by succinic semialdehyde.
dehydrogenase, and it enters the TCA cycle as succinate (Fig. 1.9) (Watanbe et al., 2002).

**Fig. 1.9**

![Metabolic pathway of GABA](image)

**Fig. 1.9: Metabolic pathway of GABA** - GAD – glutamate decarboxylase, GABA-T – gamma-aminobutyrate transaminase, SSADH – succinic semialdehyde dehydrogenase. Taken from Watanabe et al., 2002.

Majority of synapses within the mammalian central nervous system utilise amino acids like L-glutamic acid, glycine or GABA for signalling. Along with L-glutamate, acetylcholine and serotonin, GABA possesses two different types of receptors conserved across different species and phyla controlling both excitation and inhibition (Tallman et al., 2002). Molecular biology helped in discerning two receptors aiding in the GABAergic effects on ionic transmission (ionotropic) and metabolism (metabotropic). These effects are mediated by proteins in two different superfamilies. The first superfamily, GABA$_A$ receptors are a set of ligand-gated ion channels that produce fast synaptic transmission (Sieghart, 1995). The second superfamily (GABA$_B$) is slower, mediating GABA’s actions through G-protein receptors (seven trans membrane spanning receptors) (McMaster et al., 1997). Native GABA$_A$ receptors possess pentameric structure with the general composition $2\alpha$, $2\beta$ and one $\gamma$ subunit found in majority of GABA$_A$ receptors in vertebrates (Knight et al.,
1997). The major subtypes of GABA<sub>A</sub> receptors in the brain include α<sub>2</sub>β<sub>2</sub>γ<sub>21</sub>, α<sub>3</sub>β<sub>3</sub>γ<sub>21</sub>, α<sub>5</sub>β<sub>3</sub>γ<sub>21</sub> and α<sub>5</sub>β<sub>3</sub>γ<sub>21</sub>. Chromosomal deletions of particular GABA<sub>A</sub> receptor subunits showed phenotypes of craniofacial deficits, mental retardation, and epilepsy (Tallman et al., 2002). Deletion of large areas of human chromosome 15, containing α<sub>5</sub>, γ<sub>3</sub> and β<sub>3</sub> subunit genes caused a human genetic disorder called Prader Willi/Angelman syndrome (Magenis, 1996). In vivo studies with specific deletion of β<sub>3</sub> subunit in mice showed similar syndrome with cleft palate and neurologic abnormalities. These studies provide insights about the importance of certain GABA<sub>A</sub> receptors in neuronal development (Culiat et al., 1995; Homanics, 1997).

**Serotonin**

**Fig. 1.10**

**Fig. 1.10**: 5-HT receptors - 5 HT receptors are GPCR’s having seven transmembrane spanning α-helices. Binding of 5 HT activates the G-proteins which in turn initiates secondary messenger signalling pathways. The downstream effect could be either inhibitory or stimulatory (CNSforum, 2000).

**Serotonin**, plays a crucial role in regulating emotional states. Of the 14 or so mammalian serotonin receptor subtypes that have been described, at least four have been implicated in anxiety in various animal models (Lucki, 1995). Serotonin is
synthesised from the conversion of L-tryptophan to 5-hydroxytryptophan which is able to cross the blood-brain barrier and is eventually broken down to 5-hydroxytryptamine (5-HT) or serotonin. The brain serotonin receptors have been divided into a wide range of subtypes based on their pharmacological specificities, anatomical distribution and function (Fig. 1.10) (Barchas and Altemus, 1999). One of the receptor subtypes implicated in anxiety is the serotonin 1A receptor subtype (5-HT1A), an autoreceptor located presynaptically on serotonin neurons. When stimulated, this receptor inhibits the synthesis and secretion of serotonin (Tallman et al., 2002). The 5-HT1A receptor agonist buspirone exhibits anxiolytic effects in animals and is useful in the treatment of generalized anxiety disorder but not in panic disorder. In contrast to benzodiazepines, buspirone has a delayed onset of action and must be administered for up to several weeks before a significant reduction in anxiety is observed and has no sedative, anticonvulsant or muscle-relaxant activity and no significant addiction liability (Batool, 2008; Barchas and Altemus, 1999).

Other serotonin receptors potentially involved in anxiety include the 5-HT2A, 5-HT2C and 5-HT3 receptors. Antagonists for 5-HT2A receptor like ritanserin exhibit anxiolytic effects in some animal models (Critchley and Handley, 1987; Kennett et al., 1995). Likewise, blockage of the 5-HT2C receptor produces anxiolytic effect in animals (Kennett et al., 1995). In humans 5-HT2A receptor agonist m-chlorophenyl piperazine (m-CPP) has been shown to generate anxiety in control subjects and in patients with a wide variety of anxiety disorders (Barchas and Altemus, 1999). The 5-HT3 receptor antagonist ondansetron has been reported to be anxiolytic in some animal models (Costall and Naylor, 1991). The selective serotonin reuptake inhibitors (SSRIs) have proven useful for panic and obsessive-compulsive disorder. Thus, the finding that a number of drugs that are useful in panic disorder are not useful in generalized anxiety disorder and vice versa suggests that the fundamental mechanism of these processes are different (Barchas and Altemus, 1999).

**Noradrenergic neurons**

The locus ceruleus is the nucleus for 80% of brain noradrenergic neurons. This nucleus projects to multiple brain areas, including the limbic system, hypothalamus and cortex (Fig. 1.11). Activation of the locus ceruleus and the peripheral autonomic nervous system are major components of the normal stress response. Stress increases
norepinephrine in the brain aiding in fight/flight response. This increase in 
norepinephrine in the brain can be attenuated by anxiolytic agents (Barchas and 
Altemus, 1999). Noradrenergic mechanisms have been related to both panic attacks 
and post-traumatic stress disorder (Southwick et al., 1993).

**Fig. 1.11**

**Fig. 1.11: Noradrenergic pathway in GAD** - An enhanced noradrenaline 
transmission from locus coeruleus and caudal raphe nuclei has been observed in 
GAD. The locus coeruleus mediates the autonomic symptoms associated with anxiety 
viz. increased heart rate, dilated pupils, tremors and sweating (CNSforum, 2000).

**Neuropeptides**, have been implicated in the regulation of complex behaviours 
including anxiety related behaviours and psychopathology (Landgraf, 2001 and 
2005). Tachykinins such as substance P and their associated receptors have a 
widespread distribution in the brain, spinal cord, and periphery (Otsuka and Yoshioka, 
1993; Khawaja and Rogers, 1996; Longmore et al., 1997; Mantyh et al., 1989; 
Tooney et al., 2000). In addition, anatomic and physiologic evidence has also 
indicated that these peptides may affect limbic structures that are involved in the 
regulation of mood such as the amygdala, hypothalamus and periaqueductal gray 
(Culman and Unger, 1995). This notion is supported by early positive clinical findings 
using a selective neurokinin-1 (NK-1) antagonist for the treatment of depression and
anxiety (Kramer et al., 1998). Tachykinins collectively refer to small peptides including substance P (SP), neurokinin A (NK-A), and neurokinin B (NK-B). Neurokinin receptors are localized in a number of different brain areas that are implicated in anxiety, including the amygdala, hypothalamus, and locus coeruleus. Several studies targeting the neurokinin receptors have showed promising results. Several NK-1 antagonists have been reported to demonstrate anxiolytic effects in animal models such as social interaction (File, 1997), however the results are not consistent across all compounds (Griebel, 1999).

**Management of anxiety**

Management of anxiety disorders varies and depends on the nature of the disorder and individual patient characteristics (Shri, 2006). Generally the treatment involves:

- Medications
- Psychological treatment
- Alternative therapy

**Medications**

Medications will not cure anxiety disorders, but it can keep them under control while the person receives psychotherapy. With proper treatment, many people with anxiety disorders can lead normal, fulfilling lives (US Department of Health and Human Services, 2009). Commonly prescribed medications include selective serotonin reuptake inhibitors (SSRIs) which may be the first choice of medication for generalised social phobia. These drugs elevate the level of neurotransmitter serotonin, among other effects, ex. fluoxetine, sertraline, paroxetine, citalopram etc. Other medications commonly prescribed for anxiety disorders include benzodiazepines (ex. diazepam, chlordiazepoxide etc.) which facilitate inhibitory GABA transmission. Monoamine oxidase inhibitors (MAOIs) are the oldest class of antidepressant medications (US Department of Health and Human Services, 2009) (ex. phenelzine, moclobemide) that prevent the breakdown of serotonin and noradrenalin. Beta-blockers like propranolol, atenolol which reduce the ability to produce adrenaline. The common limitations of these anxiety medications or drug therapy include comorbid psychiatric disorders and increase in dose leading to unbearable side-effects.
(Cates *et al.*, 1996; Pilc and Nowak, 2005), such as allergic reactions, drowsiness, coordination problems, fatigue, mental confusion, nausea and addiction liability among others.

**Psychological treatment**

Cognitive-behavioural therapy and Exposure therapy are effectively used to treat anxiety disorders. Cognitive therapy focuses on changing patterns of thinking and beliefs that are associated with, and trigger, anxiety. The most important component of behaviour therapy is exposure. Exposure therapy includes confronting your fears to desensitise yourself to such dangers/fears that can trigger anxiety (US Department of Health and Human Services, 2009).

However, there are several alternative treatments that also help in alleviating anxiety in addition to medications. These could include:

- Meditation – beneficial to patients with phobias and panic disorders.
- Hydrotherapy – promotes general relaxation of the nervous system.
- Exercise – a natural stress buster and anxiety reliever.
- Relaxation techniques (Yoga) – include progressive muscle relaxation and controlled breathing which when practised regularly attenuate anxiety.
- Biofeedback – an effective method that uses sensors that measure physiological functions like heart rate, breathing and muscle tension and help to recognise the body’s anxiety response and learn how to control them using relaxation techniques.
- Hypnotherapy – is sometimes used in combination with cognitive-behavioural therapy. The hypnotherapist applies different therapeutic approaches to help you confront your fears while in a state of deep relaxation.
- Acupuncture – used in traditional Chinese medicine, helps alleviate anxiety (Gupta *et al.*, 2010).

**Behavioural animal models**

Studies relating to the Central Nervous System and brain are accomplished using animals as experimental models. Animal models form the backbone of preclinical research on the neurobiology of psychiatric disorders, and are employed as screening tools in the search for novel therapeutic agents (Rodgers *et al.*, 1997).
Rodents especially mice have proven to be helpful in research as mice and humans share more than 90% of their genes in common. Furthermore, animal models are particularly helpful in situations when the impact of stress cannot be studied in humans because of ethical and other reasons (Kalueff and Tuohimaa, 2004). Many of the used paradigms are ‘conflict’ tests that use both an aversive stimulus (like open space or a brightly lit environment) and a rewarding stimulus (like a familiar or nonthreatening environment, or food) (Trullas and Skolnick, 1993). The less the animals avoid the aversive stimulus, the less anxious they are. It does seem that the different tests tap into different aspects of anxiety (Belzung and Le Pape, 1994; Rodgers and Johnson, 1995). A variety of tests for anxiety have been developed of which the commonly used ones include Elevated plus maze, Light-Dark box test, Vogel’s conflict test, Open field test etc.

**Elevated plus Maze (EPM) test**

The EPM has four arms (two open and two enclosed) that are arranged to form a plus shape and elevated 40-70 cm from the floor. The model is based on rodent’s aversion of open spaces. The assessment of anxiety behaviour of rodents is done by using the ratio of time spent on the open arms to the time spent on the enclosed arms. The elevated plus maze relies upon rodents proclivity towards dark (enclosed spaces) and an unconditioned fear of heights (open spaces) (Lister, 1987).

**Light - Dark box test**

The light - dark box test in mice is based on the innate aversion to brightly illuminated areas and the spontaneous exploratory activity of mice. The apparatus comprises of a light (brightly lit) and a dark compartment separated with a partition. The distance travelled in each chamber, the total number of transitions, the time spent in each chamber and the latency to enter the light chamber are noted. The anxiolytic compounds are known to increase the total duration of time spent in the light compartment whereas the anxiogenic compounds work in the opposite way (Bourin and Hascoet, 2003).

**Vogel’s conflict test**

The Vogel’s conflict test is based on the principle that the water deprived animal is placed in the test cage with a special conductive floor grid and a drinking
water bottle with an electrically conductive nipple. The animal licks are recorded and monitored by very low electrical currents applied to the nipple that are below the animal's perception level. After a specified number of licks an electric shock is applied to the nipple and the animal can escape the shock by withdrawing from the drinking tube/nipple. The number of shocks received after treatment with the anxiolytic drug is compared with the untreated animals. The anxiolytic drugs significantly increase the number of licks and therefore the number of shocks accepted (Vogel et al., 1971).

**Open field test**

It is a generally used paradigm to assess/evaluate the locomotor, exploratory and anxiety-like behaviour in laboratory animals. The open field area/arena usually consists of brightly lit square or round area enclosed by walls with the animal usually being placed in the centre and its behaviour being recorded for a known period of time (3-15 min) (Kulkarni, 2005). It relies on the fact that the rodent when anxious stays close to the enclosed walls and measures the degree to which the rodent avoids the central area and also measures the total locomotor activity of the rodent in the apparatus.

**State-Trait Anxiety Inventory (STAI)**

STAI (Spielberger et al., 1970) is one of the most widely used self-report measures of anxiety. A means for appraisal of anxiety in research and clinical settings with questionnaires. The scores obtained are directly related with anxiety i.e. higher the score (20-80), greater the anxiety. It helps practitioners differentiate between anxiety and depression. The STAI occurs in three forms. The STAI Form X is the first version of the STAI, the STAI Form Y differentiates between temporary or emotional state anxiety versus long standing personality trait anxiety in adults, and the third form is the STAI for children (Tilton, 2008).

**Life style, stress and anxiety**

Anxiety is emerging to be a modern day liability. Physical, mental, psychological and environmental stressors which have become an inseparable part of life today are known to contribute to oxidative stress (OS) and this has been implicated in anxiety. Of late, there are reports stating that oxidative stress plays a
critical role in anxiety. Oxidative stress has been implicated in a majority of diseases affecting humans. Oxidative stress has been claimed to be a major player in the pathogenesis of lifestyle-related diseases such as atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies (Yoshikawa and Naito, 2002).

Biological oxidation occurs when molecular oxygen is added to a substance or when hydrogen is removed from a substance (dehydrogenation). Reduction occurs when molecular oxygen is removed or when hydrogen atoms are added to a substance. The major classes of free radicals in living organisms are the reactive oxygen species (ROS) and the reactive nitrogen species (RNS), which are respective collective terms for oxygen and nitrogen derived radicals, as well as some non-radicals that readily convert into radicals (Halliwell, 2006; Pacher et al., 2007).

The reactive oxygen species occur from the excitation of oxygen to form singlet oxygen ($O_2^\cdot$) or from the transfer of one, two or three electrons to form superoxide radical ($O_2^-\cdot$), hydrogen peroxide ($H_2O_2$) or hydroxyl radical (HO$^-\cdot$). These reactive oxygen species result in unrestricted oxidation of various cellular components and can lead to the oxidative destruction of the cell (Asada and Takahashi, 1987; Dat et al., 2000; Asada, 1999; Hammond-Kosack and Jones, 1996).

An antioxidant (reductant or reducing agent) can be classified as a compound capable of preventing the pro-oxidation process, or biological oxidative damage (Cao and Prior, 1998, Prior and Cao, 1999). Halliwell, (1990 and 1995) defined an antioxidant as an agent when present in low concentrations significantly prevents or delays oxidation of an oxidizable substrate. In other words, oxidative stress is defined as a “state harmful to the body, which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost” (Yoshikawa and Naito, 2002). Antioxidants have been showed to be effective anxiolytics (Vignes et al., 2006; Bouayed et al., 2007a).

However, oxidative stress is double edged and is actually useful in some instances. For example, it is known to induce apoptosis to prepare the birth canal for delivery. The biological defence mechanisms are strengthened by oxidative stress during appropriate physical exercise and ischemia. Thus, these free radicals are known to play vital roles in cellular signalling, physiological immunological responses and in mitosis. Under physiological conditions, multiple lines of defence
exist to protect against these free radicals, including the restriction of their production through the maintenance of a high oxygen gradient between the ambient and cellular environments, their removal by non-enzymatic and enzymatic antioxidants, and the reparation of oxidative damages by structural repair and replacement mechanisms (Davies, 2000; Sies, 1997). Despite the efficiency of such a versatile defence system, a certain degree of oxidative damage is inherent in aerobic life and is believed to underlie the ageing process and influence organismic lifespan (Finkel and Holbrook, 2000).

The brain is highly susceptible to oxidative stress due to its high oxygen consumption, its modest antioxidant defences and its lipid-rich constitution (Ng et al., 2008; Halliwell, 2006). Human brain utilizes 20% of oxygen consumed by the body even though this organ constitutes only about 2% of the body weight (Halliwell, 2006; Clarke and Sokoloff, 1999). In presence of oxidative stress, the lipid-rich constitution of brain favours lipid peroxidation that results in decrease in membrane fluidity and damage to the membrane proteins inactivating receptors, enzymes and ion channels (Valko et al., 2007; Halliwell, 2006). As a result, oxidative stress can alter neurotransmission, neuronal function and overall brain activity (Lebel and Bondy, 1991; Cardozo-Pelaez et al., 1999). Oxidative stress has been associated with several diseases which are specific for nervous system impairment including neurodegenerative diseases and neuropsychiatric diseases, such as schizophrenia and major depressive disorders (Valko et al., 2007; Bilici et al., 2001; Yao et al., 2001; Gingrich, 2005; Hovatta et al., 2010). As this correlation of oxidative stress and anxiety is still gaining popularity, the future promises to gain insights into their more elusive underlying mechanisms.

**Neural circuitry in anxiety**

Basolateral amygdala is primarily glutamatergic (~90%) (Carlsen, 1988; Smith and Pare, 1994) whereas the central nucleus of amygdala, which encompasses the centrolateral and centromedial nuclei, consists of ~95% GABAergic medium spiny neurons (McDonald, 1982). The primary output region of the amygdala is the centromedial nuclei (Krettek and Price, 1978a and b) which (when chemically or electrically excited) mediates autonomic and behavioural responses associated with fear and anxiety via projections to the brainstem (Davis, 2000).
Chapter 1 - Introduction and Review of Literature

The amygdala has been recognised as a key structure for processing neuronal inputs from other parts of the brain and initiating output signals to the responding nuclei and thereby generate various physiological responses including behavioural, autonomic and hormonal responses related to anxiety (Gross and Hen, 2004). Empirical studies have shown that electrical stimulation of the amygdala elicits anxiety, whereas lesion of the amygdala impairs the perception of fear (Davis, 1992). Other brain regions implicated in anxiety include cortex, hippocampus, hypothalamus and brain stem. Sensory information reaches the amygdala via projections from the thalamus, cortex or hippocampus. Internucleus interactions also facilitate transmission and integration of sensory input to the amygdala.

The sensory information flows from the lateral amygdala to the basolateral amygdala before finally reaching the central amygdala. Further, the efferents from the central amygdala go to the periaqueductal gray, brain stem and hypothalamus which respond and initiate fear-related behavioural, autonomic and hormonal responses (Fig. 1.12) (Davis et al., 1994; LeDoux, 2000). This is our body’s defence against threat/fear-related situations and a normal functioning of this circuit is critical for physiological anxiety, whereas dysfunction of this circuit will lead to pathological anxiety.

**Fig. 1.12**

**Fig. 1.12: Neural circuitry of anxiety** - Flow of information from threat perception to initiating anxiety/fear related behaviours. LA = Lateral amygdala; BLA = Basolateral amygdala; CeM = Central amygdala. Modified from, Wu et al., 2008.

**Herbal anxiolytes**

Anxiety, mood and sleep disorders are prevalent and highly comorbid psychiatric conditions that have been treated with herbal medicines since antiquity (Kessler et al., 2005; Sarris et al., 2011). Very interesting studies by Kessler et al., (2001) wherein a nationally representative sample of 2055 people interviewed,
Chapter 1 - Introduction and Review of Literature

revealed that 57% of those suffering from anxiety attacks, and 54% of those with severe depression reported using herbal medicine and complementary and alternative medicinal therapies during the previous 12 months to treat their disorder. 82 psychiatric North American inpatients hospitalized for acute care for various psychiatric disorders revealed that 44% had used herbal medicine (mainly for psychiatric purposes) during the previous 12 months (Elkins et al., 2005). These reports shed light on the importance of herbal medicines in curing psychiatric disorders mainly owing to their minimal adverse effects.

Plants are known to have enormous potential to cure ailments from time immemorial. Ayurveda and Unani are such inherited traditional systems of health and longevity that are based on herbal medicines. The ‘World Health Organization’ has approved that traditional health and folk medicine systems have proved to be more effective in health problems worldwide (Jadhav et al., 2009). However, the major hurdle in the uninhibited exploitation of herbal medicines into the regular practice of prescription is the lack of sufficient scientific data and better understanding of efficacy and safety of the herbal products (Gogtay et al., 2002).

A number of plants have been scrutinised for their anxiolytic effects. Table 1.1 gives a list of some of the widely studied plants for their anxiolytic effects.

Table 1.1: Lists herbs explored for their potential anxiolytic effects in the last 5 years

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Plant part</th>
<th>Extract</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achyranthes aspera Linn</td>
<td>Amaranthaceae</td>
<td>Leaves</td>
<td>Methanol</td>
<td>Barua et al., 2012</td>
</tr>
<tr>
<td>Actaea spicata Linn</td>
<td>Ranunculaceae</td>
<td>Roots</td>
<td>Petroleum ether, chloroform, methanol and water</td>
<td>Madaan and Sharma, 2011</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Caricaceae</td>
<td>Pulp</td>
<td>80% ethanol</td>
<td>Kebebew and Shibeshi, 2013</td>
</tr>
<tr>
<td>Cinnamomum osmophloeum ct. linalool</td>
<td>Lauraceae</td>
<td>Leaves</td>
<td>Essential oil</td>
<td>Cheng et al., 2015</td>
</tr>
<tr>
<td>Citrus aurantium subsp. bergamia (Risso) Wright and Arn.</td>
<td>Rutaceae</td>
<td>Peel</td>
<td>Essential oil</td>
<td>Saiyudhtong and Marsden, 2011</td>
</tr>
</tbody>
</table>
Chapter 1 - Introduction and Review of Literature

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Part Utilized</th>
<th>Extraction Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coriandrum sativum</em></td>
<td>Umbelliferae</td>
<td>Fruits</td>
<td>70% ethanol</td>
<td>Mahendra and Bisht, 2011</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em> DC</td>
<td>Poaceae</td>
<td>Aerial parts</td>
<td>Essential oil</td>
<td>Rodrigues de Almeida Costa <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><em>Equisetum arvenseinn</em></td>
<td>Equisetaceae</td>
<td>Stem</td>
<td>Petroleum ether, chloroform and ethanol</td>
<td>Singh <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><em>Ilex paraguariensis</em> St. Hil</td>
<td>Aquifoliaceae</td>
<td>Leaves</td>
<td>Ethanol and water</td>
<td>Santos <em>et al.</em>, 2015</td>
</tr>
<tr>
<td><em>Kelussia odoratissima</em> Mozaff</td>
<td>Umbelliferae</td>
<td>Aerial parts</td>
<td>80% ethanol</td>
<td>Rabbani <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><em>Montanoa tomentosa</em> Cerv</td>
<td>Asteraceae</td>
<td>Leaves</td>
<td>Water</td>
<td>Sollozo-Dupont <em>et al.</em>, 2015</td>
</tr>
<tr>
<td><em>Nymphaea alba</em> Linn</td>
<td>Nymphaeaceae</td>
<td>Whole plant</td>
<td>95% ethanol</td>
<td>Thippeswamy <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><em>Occimum gratissimum</em></td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>70% ethanol</td>
<td>Venuprasad <em>et al.</em>, 2014</td>
</tr>
<tr>
<td><em>Pimenta pseudocaryophyllus</em></td>
<td>Myrtaceae</td>
<td>Leaves</td>
<td>Dichloromethane fraction from ethanol</td>
<td>Fajemiroye <em>et al.</em>, 2012</td>
</tr>
<tr>
<td><em>Plumeria rubra</em> var accutifolia L.</td>
<td>Apocynaceae</td>
<td>Flower</td>
<td>Ethanol</td>
<td>Chatterjee <em>et al.</em>, 2013</td>
</tr>
<tr>
<td><em>Stachys tibetica</em></td>
<td>Lamiaceae</td>
<td>Whole plant</td>
<td>Essential oil</td>
<td>Kumar <em>et al.</em>, 2012</td>
</tr>
<tr>
<td><em>Syzygium aromaticum</em></td>
<td>Myrtaceae</td>
<td>Flower buds</td>
<td>50% hydroalcoholic</td>
<td>Tiwari <em>et al.</em>, 2014</td>
</tr>
<tr>
<td><em>Telfairia occidentalis</em> Hook. f.,</td>
<td>Cucurbitaceae</td>
<td>Leaves</td>
<td>Hydroalcoholic</td>
<td>Ajao and Akindele, 2013</td>
</tr>
</tbody>
</table>

The herb researched in the present study is called *Nardostachys jatamansi* DC., (Fig 1.13) a rhizome-bearing medicinal plant which is restricted to specialized habitats in high altitudes of the Himalaya, ranging from 3000 to 5000 m (Airi *et al.*, 2000). It belongs to plant family: *Valerianaceae* and is commonly known as Indian Spikenard. The botanical classification of the herb is as follows (Table 1.2):
Table 1.2: Botanical classification of *Nardostachys jatamansi*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Dipsacales</td>
</tr>
<tr>
<td>Family</td>
<td>Valerianaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Nardostachys</td>
</tr>
<tr>
<td>Species</td>
<td>Jatamansi</td>
</tr>
</tbody>
</table>

**Botanical name** *Nardostachys jatamansi* DC

**Fig. 1.13**

![Image of Nardostachys jatamansi DC](image)

**Fig. 1.13: Nardostachys jatamansi DC**

**Phytochemistry**

The rhizome has been reported to possess an array of chemical constituents that render myriad medicinal properties to the herb. The rhizome contains essential oil rich in sesquiterpenes and coumarins (Chatterjee *et al*., 2005). Jatamansone or valeranone is the principal sesquiterpene (Hoerster *et al*., 1977; Rucker *et al*., 1977). Other sesquiterpenes include alpha-patcho-ulanese, angelici, β-eudesemo, β-atchoulese, β-sitosterol, calarene, elemol, nardostachone, dihydrojatamansin, jatamansinol, jatamansic acid, jatamansinone, jatamansinol, oroseolol, oroselone, seselin, valeranal, nardostachyn (Chatterjee *et al*., 2000), nardosinone, spirojatamol (Bagchi *et al*., 1990), jatamol A and B (Bagchi *et al*., 1991), calarenol, seychellene,
seychelane and coumarins viz. jatamansin or xanthogalin. A new sesquiterpene acid, nardin and new pyranocoumarin have been reported. Actinidine, an alkaloid lignin and neolignins have also been reported. A new sesquiterpene acid, Nardin and new pyranocoumarin, 2', 2'-Dimethyl-3'-Methoxy-3', 4'-Dihydropyranocoumarin have also been reported (Chatterjee et al., 2005).

**Pharmacological properties**

In the Ayurvedic system of medicine, the herb is used as a medhya (brain tonic), rasayana (rejuvenates the mind), nidhrajana (promotes sleep), manasrogahna (alleviates mental diseases), pachana (aids digestion), kasawasahara (alleviates cough and breathing difficulties), kushtaghna (stops skin diseases and itching), dahaprasaḥ—mana (stops burning sensations), varnya (benefits complexion) and roma sanjanana (promotes hair growth). It is attributed as a bitter tonic, stimulant, antispasmodic, antiepileptic and antihysteric (Oleg, 1997), and is also used in treatment of nervous headache, excitement, depressive illness, menopausal symptoms, flatulence and intestinal colic.

Pharmacological activity of *Nardostachys jatamansi* DC so far reported (Table 1.3):

**Table 1.3: Pharmacological properties attributed to Nardostachys jatamansi**

<table>
<thead>
<tr>
<th>Pharmacological activity</th>
<th>Extract</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoprotective</td>
<td>50 % ethanol</td>
<td>Ali <em>et al.</em>, 2000</td>
</tr>
<tr>
<td>Cardioprotective</td>
<td>Ethanol</td>
<td>Subashini <em>et al.</em>, 2006; 2007 and 2007a</td>
</tr>
<tr>
<td>Neuroprotective</td>
<td>95 % ethanol</td>
<td>Khan <em>et al.</em>, 2012</td>
</tr>
<tr>
<td>Ischemic protective effects</td>
<td>95 % ethanol</td>
<td>Salim <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Anti-convulsant</td>
<td>95 % ethanol</td>
<td>Rao <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Nootropic</td>
<td>95 % ethanol</td>
<td>Joshi and Parle, 2006</td>
</tr>
<tr>
<td>Anti-microbial</td>
<td>Dichloromethane and methanol (1:1)</td>
<td>Kumar <em>et al.</em>, 2006</td>
</tr>
<tr>
<td></td>
<td>Essential oil</td>
<td>Mishra <em>et al.</em>, 1995; Sarbhoy <em>et al.</em>, 1978</td>
</tr>
<tr>
<td>Anti-parkinson’s</td>
<td>95 % ethanol</td>
<td>Ahmad <em>et al.</em>, 2006</td>
</tr>
<tr>
<td>Anti-depressant</td>
<td>95 % ethanol</td>
<td>Dhindra and Goyal, 2008</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Methanol fraction from water</td>
<td>Bae <em>et al.</em>, 2014, 2011</td>
</tr>
</tbody>
</table>
Thus, *Nardostachys jatamansi*, an indigenous folk medicine has been shown to be an anxiolytic herb; however there are only a few reports in support of this claim. The bio-distribution and the pharmacokinetic properties of the constituents are needed to be worked out. A number of details of the activity of the plant as well as its constituents are yet to be worked out. There is no standardized product that is available from the herb for use as anxiolytic.
OBJECTIVES

The present study has been undertaken with the following objectives:

1. To evaluate the mode of anxiolytic action of *Nardostachys jatamansi* root extract in mice.

2. To study the pharmacokinetic properties and bio-distribution pattern of the extract in animal models.

3. Development of a nutraceutical product and evaluation of the product for its efficacy.
PLAN OF WORK

1. Preparation of active constituents rich extract using 70% alcohol.

2. Screening of the extract using elevated plus maze test and open field test for anxiolytic activity.

3. Phytochemical screening, metabolite profiling and *in vitro* antioxidant activity of the most effective root extract.

4. Evaluation of the modulation of chemically-induced anxiety by *Nardostachys jatamansi* extract.

5. Evaluation of the modulation of oxidative stress-induced anxiety by the extract and evaluation of its effects on *in vivo* antioxidant enzymes and the expression pattern of oxidative stress parameters and antioxidant biomarkers in mice.

The anxiolytic properties of the extract will be evaluated using a battery of behavioural tests like Elevated plus maze, Light-Dark box test, Open field test etc.


7. Determination of the bio-distribution pattern and pharmacokinetics of the extract in animal models after radio labelling.