Basis of the study and designing aspect

Neuropharmacology is the study of the effect of compounds on nervous system. Neuropharmacology also involves understanding the actions of drugs on the functions of the brain, whether it is on single cells or behavior, is a multilevel, multifaceted process that begins with and builds upon the concept of molecular interactions (Cooper et al., 2002). Neuroprotective are the agents, which are used for treatment of neurodegenerative disorder or neural cell damage. This neural cell damage occurs in specific region (focal) or in whole brain (global). Neuroprotection is a strategy or mechanism to prevent cell loss in specific brain areas due to stroke, injury, ischemia, hypoxia etc, similar to that observes in various neurodegenerative diseases (Bhatnagar, 2006).

In today’s life, stress, strain, sedentary life style, obesity, lack of physical work and environmental pollution are becoming crucial factors in the genesis and progression of variety of CNS diseases and disorders ranging from depression, mania, anxiety, psychosis, epileptic seizures, dementia, Parkinson’s and Alzheimer’s diseases (Thakur & Mengi, 2005).

Serotonin and noradrenaline influence mental behavior patterns. Dopamine is involved in movement while acetylcholine in memory and learning. These substances are fundamental to normal brain functions. For this reason they have been center of neuroscientific study for many years. In the process of the study, new understanding has been gained of the neurochemistry of several important mental health disorders, especially schizophrenia, depression, anxiety as well as epilepsy. Such knowledge offers new opportunities for advancement in neuropharmacology, for example, the new drugs development of specific to certain receptor types that will provide relief of symptoms for many sufferers (Blows, 2002). The recent discovery that new neurons are born in certain regions of the brain each day, and may be incorporated into the existing circuitry within those regions, raises new hope for the treatment of neurodegenerative and other neuropsychiatric disorders.

More than 20% of population suffers from CNS disorders at some time during their life. The WHO predicts CNS disorders will become the leading cause of premature death or disability worldwide by the year 2020.
Approximately two-third of the patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Then, the medical need for newer, better tolerated and more efficacious treatments remains high. At present many therapeutic agents are available to treat these disorders, but the major limitations of these drugs are their side effects and high cost of modern treatment; indicates a dire need for the development of new safer drugs and alternative strategies for the prevention and treatment of many diseases and disorders (Mora et al., 2006).

2.1 Conventional Drugs:

During 20\textsuperscript{th} century the progress in chemical techniques and with the growth of pharmaceutical industry, crude drugs were replaced gradually by chemical (synthetic) drugs, however contending with various mechanism in human body to prevent their excessive actions, have a single mode of action and may cause severe adverse reactions when given in large doses or over long period of time or some time differ with individuals. The synthetic drugs can produce remarkable life saving results in acute diseases but cannot be used in the treatment of chronic diseases (Sharma, 2003).

2.2 Limitations of conventional drugs:

For the treatment of several mental disorders like depression, psychosis, cognitive dysfunction, anxiety, epilepsy etc. chronic administration of drugs is necessary. During the last two decades, psychoactive drugs have been increasingly recognized as most effective in the management of anxiety, stress and psychomotor disorders. However, the prolonged use of tranquilizers and psychotropic drugs leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Furthermore, such agents relieve the symptoms primarily and offer a palliative relief of a temporary nature (Thakur and Mengi, 2005). Synthetic agents are available for the treatment of nervous system disorders like anxiolytics, hypnotic, anticonvulsant and antipsychotic etc. They are effective in counteracting disease induced ill-effects. But, the problems of tolerance and physical dependence exhibited by Benzodiazepines, on prolonged use, limits their utility. Furthermore, these drugs have adverse effects on the fetus during pregnancy and on the neonate during lactation. Neuroleptic agents cause several adverse effects such as extrapyramidal symptoms, sedation, hypotension, agranulocytosis etc (Tripathi, 2008).
2.3 Importance of Herbal Medicines:

The two prominent system of medicine with history of existence for thousands of year include Ayurveda and Chinese system of medicine. Ayurvedic medicines use largely herbal and herbo-mineral preparations to treat the diseases such as cancer, cardiovascular diseases, diabetes, hepatitis and asthma.

Ayurveda takes a holistic view of human disease. It views any disease as a dysfunction of the whole body rather than of a single organ or physiological process. Most of the Ayurvedic drugs therefore are likely to act on a number of dysfunctions of the body involving a number of organs and functions. A major discovery of the past two decades in the field of neurosciences has been the elucidation of behavioral, neurobiological and cellular basis of many CNS disorders. Despite several advances in the health care system, numerous new diseases have emerged. Considering the inherent weakness and limitations, allopathy by itself could not tackle the plethora of problem posed by emergence of new diseases. Many newer drugs which have developed recently represent a real progress in the treatment of non responders and refractory patients. However, the problem of adverse effects has also not been circumvented completely and approximately two/third of the patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Hence, search should continue to develop newer, more effective and safer neuroprotective agents for the treatment of various CNS disorders (Kasture, 2002). In search for new therapeutic agents for the treatment of CNS disorders, medicinal plant research, worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Many of drugs are derived from natural products.

Mental ailments are heterogeneous diseases and will probably require a selected arsenal of drugs with different modes of action for successful treatment of their various manifestations. Although alternative treatments are increasingly being used to alleviate CNS disorders, strong evidence to recommend the use of herbal medicines for several illness is still scarce (Hellion-Ibarrola, 2006).

According to recent estimation of WHO, 70-80% of the world population relies on traditional system of medicine, mostly herbal medicines for their primary healthcare need (WHO, 2001).
Plant based products have been in use for medicinal, therapeutic or other purposes right from the dawn of history. Drugs used in traditional systems of medicine are all crude drugs. Until 19th century; even medicine depends largely on crude drugs. A herb or crude drug is a complex potpourri of compounds, some beneficial, some harmful, some vitamins and some even toxic—but all integrated under a certain (natural) rule to make a crude function in the same way as a single chemical agent. This crude drug, thus act as a single chemical agent without any or much side effects unlike synthetic. Thus, crude drugs have substances amalgamated in fashion where one chemical counter balances the effects of other, ultimately to give only beneficial effects. It is the reason why generally natural medicines induce fewer side effects than conventional western drugs (Handa & Kapoor, 2004).

Source of these crude drugs and herbal products are the medicinal plants. Ayurveda is also mainly based on these medicinal plants. Most of medicinal plants are easily available and within the therapeutic reach of mankind because of which this oldest system of medicine, Ayurveda is still surviving and serving the sufferings of human being since thousands of years. All of these herbal drugs can be used in both ways, viz. in single form or combined form. To make therapeutically more effective, easily administrable, palatable, digestible, less toxic and more preservable, these drugs are processed in different forms, viz., decoction, infusion, powder, juice etc (Mhaskar, et al., 2000).

In recent times, focus on plant research has increased globally and the evidence collected shows the immense potential of medicinal plants. Various plant materials are used to promote health and in treatment of various disorders as advocated in Ayurveda. In medicine also, plants occupy a very significant place as raw materials for some important drugs. The medicinal herb is a bio-synthetic laboratory as it contains a number of chemical compounds like glycosides, alkaloids, sterols, resins etc. these compounds exerts therapeutic effects and account for medicinal properties of the herbs. Plants are called as the ‘Sleeping Giant’ for the drug development because they have given important lead in drug research.
2.4 Need of new research:

In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs or to confirm the claim made about them in the official books of Ayurveda. Herbal medicine is an example of long-standing tradition that offers a unique insight onto comprehensive approach to CNS disorders (Une, et al., 2001).

People from different regions of the world have used herbal medicines to alleviate disorders for many years. In addition, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade. An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines, and also there are a large number of herbal medicines whose potential has been assessed in a variety of animal models. In fact, these models have contributed to the screening of new psychopharmacological tools and to the understanding of their biological activity (Hellion-Ibarrola, et al., 2006). There has been renewed interest in exploring the herbal medicines for the management of number of psychiatric disorders.

Herbal medicine is one of the alternatives people are turning to and herbal remedies will be with us for long time. Medicinal plants are important for pharmacological research and drug development not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacological active compounds. Despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been studied for possible medicinal applications. Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparation containing them. An innovative research effort to define the advantages of traditional systems of medicine with respect to their safety and efficacy could result in a better utilization of these complementary systems of medicine.

The discovery and development of novel therapeutics for central nervous system (CNS) disorders is dependent on contributions from a wide range of different fields of expertise-from initial reductionistic, molecular efforts to identify a specific target mechanism and to discover therapeutic agents with the desired mechanism of action, to assess the potential efficacy and safety of those
therapeutic agents at the behavioral level in animal models. In keeping with the
goals and objectives the process of preclinical research and development is very
important to develop novel treatments.

Many CNS disorders remains a mystery, with its cause and cure not yet
found, there's considerable excitement and hope about the new findings that are
unfolding in numerous research settings. There opened a new dimension in the
treatment of CNS disorders with the help of traditional medicine, which has
proved to be effective in studying the deadly disease in animal model.

2.5 Criteria for selection of plant:

In literature survey of traditional medicinal plants, it was observed that
plants taken for the present research work possesses wide range of CNS
activities.

*Brassica nigra*, Linn commonly known as black mustard belongs to the
family *Brassicaceae* (also called *Cruciferae*). The mustard family (*Brassicaceae*)
is larger than most as over 40 genera, including more than 200 wild and
cultivated species, are in the U.S. and Canada alone. *Brassica nigra* Linn., black
mustard, is an annual herbaceous plant in the *Brassicaceae* (the cabbage or
mustard) family that originated in the Middle East and is now widely cultivated
as a primary source of the mustard seeds used in making the condiment sauce,
table mustard. Young mustard plants and leaves may be eaten as a salad or
cooked green, but the plant is primarily used for its seeds. Black mustard seeds
are typically ground and mixed with water or vinegar to make prepared mustard,
which is one of the most important condiments in European and North American
cuisine. It is used as an ingredient in numerous sauces and salad dressings, and is
popular for eating with sausages (such as the German bratwurst and American
hot dogs). Black mustard is one of several *Brassica* species that are used as
winter cover crops. (Callihan B, et al).

*Brassica* vegetables are a family of 6 agriculturally important species consumed
in high quantities throughout the world. Cruciferous vegetables are a good source
of many health promoting and potentially protective phytochemicals including
folic acid, phenolic, carotenoids, selenium, glucosinolates and vitamin C (Kumar
S, et al., 2012). The different parts of *Brassica nigra* Linn. has traditionally used
as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulant
and vesicant apart from the reported use plant is having application in allied field
such as kitchen, phytomedicine, general medication for its medicinal value (Vinyas M, et al, 2012). The seed extracts of Brassica nigra Linn was reported as antidiabetic, antihyperlipidemic, antibacterial, anthelmentic, antiepileptic activity. The leaves hot with a pleasant taste increase the bile, vermicide, good for throat complaints and seeds are bitter, hot and acrid used to cure enlargement of the spleen and dispel fever, increase the bile, remove cough, tumors, increase appetite, cure skin diseases, itching and destroy external parasites is mentioned in Ayurveda. Poultice of seed are used counter irritant in several complaints of nervous system, pneumonia and an emetic in narcotic poisoning (Nitin Kumar U, et al, 2011).

Amaranthus spinosus Linn., (Amaranthaceae) commonly known as spiny amaranth or pig weed. It is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands. Its juice was used by tribal of Kerala, India to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2-3 days to cure jaundice (Hema, et al., 2006). In Indian traditional system of medicine (Ayurveda) the plant is used as antipyretic, laxative, diuretic, digestive, antidiabetic, anti-snake venum, antileptotic, CNS disorder, blood diseases, bronchitis, piles and anti-gonorrheal (Kirtikar & Basu, 1987). The Chinese use A. spinosus as traditional medicine to treat diabetes and seeds used as poultice for broken bones. Some tribes in India apply A. spinosus to induce abortion (Grubben & Denton, 2004). A. spinosus is also used as reported to possess anti-inflammatory (Olumayokun, et al., 2004), antimalarial (Hilou, et al., 2006), Antiandrogenic (Murgan, et al., 1993b), immunomodulatory (Tatiya, et al., 2007), anti-diabetic, anti-hyperlipidemic, spermatogenic activities of stem (Sangameswaran & Jayakar, 2008) and effect on hematology (Olufemi, et al., 2003) and biochemical changes in Epididymis (Murgan, et al., 1993). The betalains in stem bark of A. spinosus were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides (Hilou, et al., 2006; Ibewuike, et al., 1997; Rastogi & Mehrotra, 1999; Stintzing, et al., 2004; Ashok Kumar, et al., 2008). It also contains amaranthoside, a lignan glycoside, amaricine, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline (Blunden, et al., 1999; Azhar-Ul Haq, et al., 2006). Betalains are well known for their antioxidant, anticancer,
antiviral and antiparasitosis properties (Kapadia, et al., 1995; Kapadia, et al., 1996; Patkai, et al., 1997).

*Annona squamosa*, Linn., *(Annonaceae)* commonly known as custard apple, sugar apple, sweet soup, sour soup and tatatopa is a well-known fruit for its nutritional composition as well as for its medicinal properties. Custard apple tree is a tropical deciduous tree distributed throughout India. Leaves of *Annona squamosa* are used to treat ulcer and wounds. Major Phytochemicals present in this fruit are alkaloids, volatile matter, glycosides, flavonoids, long chain fatty acids, steroids, acetogenins, phytosterols, tannins, cyclic ketones, sterpenoids and phenolic compounds. The edible portion of this fruit contains proteins, fats, carbohydrates, crude fibers, minerals, calcium, phosphorus, iron, magnesium, copper, ascorbic acid, niacin and nicotinic acid. The glycemic index of fruit is low due to its low sugar content. Each and every part of the custard apple tree such as bark, leaf, stem, seed are used for therapeutic purposes (Pomer KW, et. al., 2009). Herbal drugs developed from plants are known for their safety and efficacy as compared to synthetic drugs. In *In-vivo* studies, it was concluded that custard apple seeds were 300 times more effective than taxol, an anticancer drug (Chenyoungh, et. al., 2012). Custard apple fruit is a new super fruit of the 21st century possessing antiobese, antidiabetic, antimicrobial and anticancer properties (Parthasaradhi, et. al., 2004). Both ethanol and aqueous leaf extracts of *Annona squamosa* showed good antidiabetic properties in type 1 and type 2 diabetes (Vinut, et. al., 2012, Gupta, et. al., 2005, Ragavendra, et. al., 2011). Another component of this fruit is acetogenin. The acetogenin compounds are very unique to the *Annonaceae* family, which are likely to have anticancer activity (Ragasa, et. al., 2012).

All plants are claimed as a nerve tonic that releases stress, negativity and tension in heightened activity. Nevertheless there is no scientific evidence about potential effects of these plants in animal models of psychiatric diseases. In spite of the reported pharmacological properties of *Brassica nigra, Amaranthus spinosus* and *Annona squamosa* in a variety of models, there are less major investigative reports available pertaining to its neuropharmacological effects, which provided a way to study neuropharmacological profile of the plants and explore these plants for nervous system disorders.
In this study, the different parts of *Brassica nigra*, *Amaranthus spinosus* and *Annona squamosa* extracted with different methods, solvents and screened them for different neuropharmacological activities. Major aim of our study was to evaluate **Phytochemical Investigation and Pharmacological Exploration on some Medicinal Plants Extracts for its Psychotropic & Anxiolytic activity** with an objective phytochemical investigation, standardization, characterization for presence of phytoconstituents, toxicity studies and pharmacological exploration of selected plants parts extracts, fractions for psychotropic and anxiolytic activity.

### 2.6 Plan of work

1. Collection, identification and authentication of plants
   a. *Brassica nigra* (Seeds)
   b. *Amaranthus spinosus* (Leaves)
   c. *Annona squamosa* (Fruits pulps)
2. Processing of crude drug
3. Pharmacognostic evaluation of plant material
4. Extraction of collected plant materials by suitable method and solvents
5. Phytochemical screening, TLC and HPLC fingerprinting of plants extracts.
6. Phytochemical standardization of plants extracts by total polyphenols and total flavonoids
7. Pharmacological Screening of plant extracts for-
   a. Evaluation of anti-oxidant screening by DPPH scavenging and %RRI
   b. Acute and Sub-acute toxicity study.
   c. *In vitro* Pharmacological screening of plant extracts on dopamine induced contractions of isolated rat vas deferens, serotonin induced contractions of isolated rat fundus and acetylcholine induced contractions of isolated goat tracheal chain preparation.
   d. *In vivo* Pharmacological screening of plant extracts for
      - Evaluation of Anxiolytic activity by
        - *Elevated plus maze (EPM)*
        - *Light and Dark paradigm (L/D)*
Chapter 2

Designing Aspect

- Evaluation of Antidepressant activity by
  - Forced swim test
  - Tail suspension test
- Evaluation of Anticonvulsant activity by
  - Pentylentetrazol (PTZ)-induced seizures
- Evaluation of Antipsychotic activity by
  - Lithium-induced head twitches
  - Haloperidol-induced catalepsy
- Evaluation of Antiaggression activity by
  - Foot shock-induced aggression (FSIA)

8. Fractionation of selected extracts
9. Phytochemical Screening, TLC and HPLC fingerprinting of fractions.
10. Phytochemical standardization of fractions by total polyphenols and total flavonoid content.
11. Pharmacological Screening of selected fractions for
    a. In vitro screening like antioxidant activity and dopamine-induced contractions of isolated rat vas deferens, serotonin-induced contractions of isolated rat fundus and acetylcholine-induced contractions of isolated goat tracheal chain preparation.
    b. In vivo screening for anxiolytic, antidepressant, anticonvulsant, antipsychotic and antiaggression activity with various models
12. Interpretation of data and its presentation.