In the 21st century, medicinal herbs are gaining importance in mainstream healthcare as greater numbers of people seek relatively safe remedies and approaches to healthcare. The demand for herbal medicines, herbal health products, herbal pharmaceuticals, nutraceuticals, food supplements and herbal cosmetics etc. is increasing globally due to the growing recognition of these products as mainly non-toxic, having in the main less side effects, better compatibility with physiological flora, and availability at affordable prices (Dubey et al., 2004, Sharma et al., 2008). Traditional medicinal (TM) systems advocated the use of medicinal plants and an integral component of traditional medicinal systems. Herbal medicines have been used and documented in Indian, Chinese, Egyptian, Greek, and Roman medicinal systems for about 5000 years as suggested by earliest as well as traditional literatures. The transcripts of classical TM systems in India include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita. Folk (tribal) medicines are also important sources for the indigenous healthcare system. India has been known to be a rich repository of medicinal plants from ancient civilizations. The forests of India are the hotspots as principal source of large number of medicinal and aromatic plants (De et al., 2010, Kamboj, 2000, Mukherjee, 2008). Many of the available bioactive drugs are from medicinal plants only. Medicinal plants are still now considered as the major source for new bioactive drugs. Research into medicinal plants, isolation of bioactive constituents and pharmacological screening can assist us to find new therapeutically active drugs or lead. In the codified systems such as Ayurveda, Siddha and Unani traditions and in folk medicine of India, there are estimated more than 6000 higher plant species mentioned (Ved and Goraya, 2008). With its rich wealth of herbs, India is on the threshold of an herbal revolution and is able to supply medicinal plant resources to meet the increasing global demand.

According to a Smithsonian statistic, 82% of the world’s population uses herbs as a primary system of healing. Herbal medicine is so powerful and effective that it has survived for thousands of years. By contrast, conventional medicine is less than 100 years old. Not only is herbal medicine an ancient, time tested and effective system of medicine, but it has evolved with humans. It is a living tradition which changes and grows with people. In fact, the history of medicine is the history of herbal medicine. From the earliest times, herbs have been prized for their healing abilities, and today we still rely on the curative properties of plants in many of our medicines. Over the centuries, societies around the world have developed their own traditions using medicinal plants.
TM occupies an important place in the healthcare systems of developing countries. The World Health Organization (WHO) estimated that more than 80% of healthcare needs in these countries are met through traditional health care practices. The people in developing countries depend on TM, because it is cheaper and more accessible than orthodox medicine (Sofowora, 1982; WHO, 2002; Tabuti et al., 2003). TM is also more acceptable because it blends readily into people’s socio-cultural life. TM is the total knowledge, skills and practices based on the theories, beliefs and indigenous cultural experiences, whether explicable or not, used in the maintenance of health, diagnosing, preventing or eliminating physical, mental or social diseases. Such knowledge may rely exclusively on past experience and observations handed down from generation to generation, verbally or in writing (Sofowora, 1982; WHO, 2002; Tabuti et al., 2003).

The TM system is holistic in that its application usually covers the mind, body and soul (WHO, 2002). The concept includes mystical and magical rituals, herbal therapy, psychiatry and other treatments, which may not be explained by modern medicine. Studies suggest that this therapy is applied to conditions such as cancer, arthritis, chronic back pain, gastrointestinal problems, chronic renal failure, eating disorder, physical, mental or social disease and so on (Sofowora, 1982). For all but the last 50 years, humans have relied almost entirely on plants to treat all manner of illness, from minor problems such as coughs and colds to life threatening diseases. Scientific inquiry is now validating the effectiveness of traditional herbal therapy.

Herbal formulations, which have attained widespread acceptability as therapeutic agents in India, include nootropics, neuroprotective, antidiabetic, hepatoprotective agents, lipid lowering agents and many more. The pharmacological effects of many plants have been studied in various laboratories, whereas there are many limitations regarding the safety and efficacy of these preparations. Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substance on living organism. In order to establish the safety, efficacy and dose determination of new drug, toxicological studies are very essential experiment in animals like mice, rat, guinea pig, monkey, rabbit etc under various conditions of drug. Medicinal plants continue to draw wide attention for their roles in case of mild/chronic diseases, and herbal medicines have received an increasing interest as documented by the numerous and rigorous published studies (Mauri et al., 2000).
A number of medicinal plants have been shown to offer an alternative to synthetic drug substances in preventing and treating some chronic and mild diseases, provided that they are of adequate quality and properly used. Quality control is never easy because medicinal plant extracts are complex mixtures of different compounds and often their identity is only partially known. Among the active principles present in medicinal plants, flavonoids, terpenes and caffeic acid derivatives have attracted a great interest in scientific research (Mauri et al., 2000). The use of plant medicines in the treatment of various ailments, including central nervous system (CNS) disorders, is an age long practice.

It is important to note that plant medicines are also gaining popularity in developed countries. Herbal medicine is currently enjoying a revival in popularity in the Asia and in fact it is the primary form of medicine in many parts of the world (Williamson et al., 1996). With the great reliance on this type of medicine, it becomes pertinent to search for potent, effective and relatively safe plant medicines as well as scientific validation of the success claims about plants already in use by traditional medicine practitioners in order to enhance their safety and efficacy. These are some of the problems making this alternative healthcare system less acceptable, especially by orthodox medicine practitioners. Although many TM or complementary, alternative medicine (CAM) therapies have promising potential, and are increasingly used, many of them are untested and their use is not monitored. As a result, knowledge of their potential side effects is limited. This makes identification of the safest and most effective therapies and promotion of their rational use more difficult (WHO, 2002). According to some authors in the field of plant medicines, the interpretation of data in terms of potential therapeutic application of plant extracts, including pure extracts, must depend on the total pharmacological profile of the extracts (Atta and Alkofani, 1998; De Sarro et al., 1999; Rabbani et al., 2003). Furthermore, there is very little scientific data on traditional medicinal plants used for CNS disorders in this country. Hence, the present study aims to investigate some of the CNS effects of *N. oleander* flower such as anti-anxiety, antiepileptic and Alzheimer Disease (AD).

### 1.1. Herbal Toxicity

Many of the drugs first used in modern medicine are extracted from plants these are known as herbal medicines. Herbal medicines are popular remedies for diseases used by
a vast majority of the world’s population. Herbalists use whole plants and traditional physicians use purified ingredients derived from plants. Traditional physicians and scientists generally believe that, if a plant has any medicinal value at all, it is because it contains one "active" ingredient that must be isolated and purified. Herbalists believe results are better when the whole herb is used, because different components of the plant act synergistically. There are risks and benefits to both approaches. If an "active" ingredient is isolated, then it can be given in a more concentrated form. This means that the effects, both therapeutic and toxic, will be exaggerated. On the other hand, if the whole plant (or leaves, or roots, depending on the plant) is used, the concentration of the active ingredient may or may not be sufficient to produce the desired therapeutic result, but also the chances for toxicity are decreased. Herbalists also believe that combining herbs improves efficacy and reduces adverse effects. Even without isolating the active principle, some herbal products are very toxic.

The pharmacological effects of many plants have been studied in various laboratories, whereas there are many limitations regarding the safety and efficacy of these preparations (Kuruvilla A., 2002). Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substance on living organism (Alam AHMK et al., 2006). In order to establish the safety and efficiency of new drug, toxicological studies are very essential experiment in animals like mice, rat, guinea pig, monkey, rabbit etc under various condition of drug. No drug is used clinically without its clinical trial as well as toxicity studies (Alam AHMK et al., 2006). Toxicological study helps to make decision whether a new drug should be adopted for clinical use or not (Anisuzzaman, ASM et al., 2001).

1.2 Plant Profile

_Nerium oleander_ Linn. (Khare, 2007)

**Family**: Apocynaceae.

**Habitat**: Native to Mediterranean

**Region**: Grown in Indian gardens.

**English**: Red Oleander, Rose Bay.

**Unani**: Surkh Kaner.

**Action**: Root—resolvent and attenuant. A paste of the root is externally applied to haemorrhoids and ulcerations in leprosy. Paste of the root bark and leaves is used
in ringworm and other skin diseases. An oil extracted from the root bark is used in skin diseases of scaly nature. Leaves—cardioactive (digitalis-like effect) and diuretic, anti-inflammatory, antifungal, insecticidal, toxic. (The white- and red-flowered varieties are equated with *Nerium oleander*; both possess similar properties. The yellow-flowered variety is equated with *Thevetia peruviana*.)

**Key application:** Leaf—including among unapproved herbs by German Commission E. Positively inotropic and negatively chronotropic actions have been mentioned; the use of leaf for diseases and functional disorders of the heart, as well as for skin diseases has been indicated. The leaves and roots gave a number of active principles including glycosides, terpenoids, sterols and other compounds. Cardiac steroids isolated from the leaf, include oleandrin, gentiobiosyl oleandrin, odoroside. The stem contained alanine arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. A polysaccharide (2.3%), containing galacturonic acid, rhamnose, arabinose and galactose have been isolated from leaves. Neutral fraction from leaves at low doses caused marked suppression of locomotor activity. Aqueous extract of leaves showed significant antibacterial activity against *Pseudomonas aeruginosa*. The leaves also showed insecticidal activity.

1.3. An Overview: *Nerium oleander* Linn.

*Nerium oleander* Linn. (Family: Apocynaceae) is a traditional Ayurvedic herb and also known as Kaner in Hindi and Surkh Kaner in Unani. In Ayurveda there are several other names indicating this plant such as Karavira, Viraka, Ashvamaaraka, Hayamaaraka, Gauripushpa, Divyapushpa, Shatakumbha, Siddhapushpa (white-flowered variety) Raktapushpa, Raktaprasava, Ravipriya (red-flowered variety) (Khare, 2007, Chopra et al., 1956). In English it is known as Red Oleander or Rose Bay. The common oleander is an ornamental evergreen shrub, omnipresent in temperate and subtropical regions (Manna et al., 2000, Ellenhorn and Barceloux, 1988). The plant is also widely grown in Indian gardens. The plant is also widely found in humid and coastal areas including Assam, Arunachal Pradesh, Himachal Pradesh, Rajasthan, Uttar Pradesh and Tamilnadu (Chopra et al., 1956, Khare, 2007). The plant is densely branched, dwarf and intermediate growing 4-30 ft tall. *N. oleander* branches have a smooth pale green to light gray bark that produces milky juice. Each stem node has two or three narrow elliptic
leaves with entire smooth margins, 4-12 inches long and half to one inch wide, with short petioles. The foliage is evergreen and leathery, dark green above and light green below. *Oleander* flowers grown throughout the year but the best growth have been seen in the warmer months (April-July). Clusters of flowers are developed on the tips of the branches and are about 3-4 inch. Flowers may be Pink, Red, Salmon, Yellow, White and Bicoloured. The Petal of each flower is about one inch long. Flowers may be single with five petals, intermediate with two set of petals or double with two or more Whorls of petals. Fruits of *Oleander* are long narrow pods usually occurring in pairs. *(Ornamentals and Flowers, 1997).*

Preparations of oleander have been used for centuries as folk and indigenous remedies for various ailments including indigestion, malaria, leprosy, mental or venereal diseases and as abortifacient *(Shaw and Pearn, 1997).* *N. oleander* is also used indigenous as a cardiac tonic, diuretic, molluscicide and insecticide and for the treatment of epilepsy, snake bites and skin conditions *(Al-Yahya et al., 2000).* Previous studies reported the use of oleander extracts for cardiac insufficiency which was mainly attributed to the cardiac glycosides within this plant *(Langford and Boor, 1996).* Oleandrin is one of the most prominent secondary compounds of *N. oleander.* The plant is also known to be toxic against a wide range of tumor cells *(Manna et al., 2000).* The plant is also documented as a potential vegetable source of antioxidants *(Moure et al., 2001).* According to the previous phytochemical works, the leaves of *N. oleander* contain two novel cytotoxic pentacyclic triterpenoids *cis*-karenin (3β-hydroxy-28-Z-p-coumaroyloxy-urs-12-en-27-oic acid) and *trans*-karenin (3-β-hydroxy-28-E-p coumaroyloxy-urs-12-en-27-oic acid) *(Siddiqui et al., 1995)* as well as two new cardiac glycosides, kancroside and ncriumoside *(Siddiqui et al., 1986).* Another study also revealed the presence of Oleandrin, Folinerin, Adynerin, Digitoxigenin Cardiac glycosides in oleander *(Bandara et al., 2010).* Seeds of *N. oleander* were reported to contain about 12% of ∆9-hydroxy-18:1∆12 (isorinicoleic acid) *(Gummeson et al., 2000).* Methanolic extract of the leaves of this plant was found as anticonvulsant, CNS depressant and analgesic *(Zia et al., 1995).* As far our literature survey is concerned, extracts from different parts of this plant have been reported to possess biological activity and various phytochemicals have been isolated from different parts of this plant including some potential hepatoprotective constituents *(Oleanolic acid) (Liu, 2005),* while no report has been published in reference to the flowers of this plant. Preliminary screening also suggested potent lipid
peroxidation inhibitory activity of the methanolic extract of the flowers of this plant under investigation.

Moreover all parts of the oleander plant are poisonous to man, animals and certain insects. Inhalation of smoke or ingestion of sap or honey produced from oleander has resulted in poisoning of humans and animals. Despite their toxicity oleanders have been used in the past as an abortifacient, a treatment of ‘dropsy’(congestive heart failure), leprosy, malaria, ringworm, indigestion, venereal disease and even as a suicide instrument (Morton, 1977). Ingestion, Inhalation or contact of mucus membranes with oleander or oleander extract can cause adverse effects. Contact dermatitis may result from skin contact to the sap of freshly cut leaves of oleander (Dorsey, 1962). Irritation of contacted membranes, buccal erythema, nausea, vomiting, increased salivation, abdominal pain, diarrhoea, headache, altered mental status, visual disturbances, mydriasis, peripheral neuritis and cardiovascular symptoms have all been reported as a result of oleander ingestion. (Haynes et al., 1985: Kingsbury, 1964: Yarbrough, 1983: Ellis, 1978: Shaw and Peam, 1979: Schwartz et al., 1974: Osterloh, 1982).

The visual disturbances associated with oleander toxicity are rather peculiar, with victims sometimes reporting seeing yellow and green colors intermixed with geometric patterns surrounding objects in the visual field (Mack, 1984). All Parts of plant are reputed as therapeutic agent and has been used in folklore in a variety of ailment (Dymock et al., 1891). The leaves and bark are used as heart tonic, diuretic, expectorant, diaphoretic and emetic (Chopra et al., 1956). Roots boiled in water are considered helpful when applied externally in skin complaints (Dymock et al., 1891) herpes and ringworm infections (Nadkarni, 1976). Leaf juice in very small doses is given in snake and other venemous bites (Nadkarni, 1976). Juice of young leaves is effective in ophthalmia with lacrimation. Infusion of the leaves is abortive (Perry, 1980). Root paste is used externally in hemorrhoids (Chopra et al., 1956), various types of cancer (Dymock et al., 1891), Ulceration and leprosy (Chopra et al., 1956). Alcoholic extract of its various parts possess antibacterial activity (Manjunath 1948). Oil extracted from roots bark is a cure for skin diseases (Dymock et al., 1891). The plant has been extensively studied both phytochemically and pharmacology and number of compounds with variety of activities has been isolated. More than 200 compounds are been isolated from the different parts of the plant. The N. oleander mainly contains Alkaloids, Glycosides, Steroids, Terpenoids and other compound. The leaves, flowers and stem
bark of *N. oleander* possess Cardiotonic properties. The first cardiotonic principle in the plant named “Oleandrin”. *(Wealth of India, 1952)*. Leaves contain glycoside Oleandrin, nerifolin, adynerin and neriantin. Apart from the Oleandrin a series of cardiac and other steroidal and no steroidal glycosides are also present *(Siddiqui S et al., 1990)*. A number of pharmacological activities have been attributed to *N. oleander* few of them are as: Antimicrobial activity *(Hussain MA et al., 2004; Sulaman KD et al., 1993)*, Ototoxicity, Sedative-Hypnotic *(Haque AZ et al., 1993)*, Immunomodulator *(Al-Farwachi, 2007)*, anti-inflammatory activity, antinociceptive activity *(Erdemoglu et al., 2003)*, Antitumor activity *(Smith et al., 2001)*, Antidiabetic, cardiotonic effect *(Yassin MM et al., 2007)*, CNS Depressant, Locomotor activity *(Zia A et al., 1995)*, Diuretic *(Rougier G et al., 1953)* and Antileukemic *(Turan N et al., 2006)*.

**Figure 1.** Shrub of *Nerium oleander*  
**Figure 2.** Flower of *Nerium oleander*

**1.4. The study**

To establish the pharmacological profile of the *N. oleander* flowers, it is essential to assess its toxicity so that standardization of dose for therapeutic explorations can be done. As far our literature survey is concerned, extracts from different parts of this plant have been reported to possess biological activity and various phytochemicals have been isolated from different parts of this plant including some potential hepatoprotective constituents *(Oleanolic acid) (Liu, 2005)*, while no report has been published in reference to the flowers of this plant. Preliminary screening also suggested the flowers of this plant under investigation as potent antioxidant, lipid peroxidation inhibitor as well as a significant cognition enhancer. Any plant desired to be used as medicine in human should be always toxicologically evaluated and the effect on CNS should also be monitored to rule out the possibility of any neurotoxicity. Therefore the study is also
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**Introduction**

Directed towards evaluating the neuropsychopharmacological profiling of the flowers of *N. oleander* along with the toxicity profiling using OECD guidelines. In this present research work an attempt has been made to evaluate the hepatoprotective activity, the potential of this plant in reversal of dementia of AD in a rat model as well as antioxidant profile of *N. oleander* flower extract with a view towards elucidating the probable mechanism of action by employing various *in vitro* and *in vivo* methods.

1.5 Central Nervous Disorders

Brain function is the single most important aspect of physiology that defines the difference between humans and other species. Disorders of brain function, whether primary or secondary to malfunction of other systems, are a major concern of human society, and a field in which pharmacological intervention plays a key role. Psychological insufficiency or overload without any biochemical evidences includes stresses in our day to day life due to pressure for work commitments, family problems etc which leads to anxiety, depression, insomnia and other behavioural disturbances. When this kind of behavioural disturbances continues for a long time these leads to major psychological dysfunction and contributes to major organ dysfunctions which include metabolic diseases like diabetes and hypertension etc.

Neuropsychopharmacology links the frontiers of basic neurosciences to the treatment of neurological and psychiatric diseases. On one level, this scientific field seeks to understand how drugs can affect the CNS selectively to relieve pain, heighten thought, induce sleep, reduce fever or appetite, suppress disordered movement, or prevent seizures. Notably, this is the field that seeks to understand how drugs can treat anxiety, mania, depression or schizophrenia without altering consciousness. The main underlying concept of Neuropsychopharmacology is that drugs which influence behaviour and improve the functional status of patients with neurological or psychiatric diseases act by enhancing or blunting the effectiveness of chemical transmission at the site of principal interneuronal communication, the specialized chemical junction termed synapses.

Diagnosis in psychiatry, as much of medicine is based mainly on identifying recognized patterns of subjective symptoms. These symptoms involve abnormalities of behavior, mood, perceptions, thinking and intellectual function
1.5.1 Classification of psychiatric disorders

Currently there are two main classification systems in use. These include *The American Psychiatric Association’s Diagnostic and Statistical (4th edition) Manual* usually abbreviated to DSM 4 and WHO international classification of disease (10th edition) known as ICD-10.

The WHO classification of psychiatric disorders is as follows:

a. Organic
   
   (1) Acute: e.g.; delirium
   
   (2) Chronic: e.g.; dementia

b. Substance abuse

c. Schizophrenia and delusional disorders

d. Affective (mood) disorders
   
   (1) Depression
   
   (2) Mania
   
   (3) Bipolar disorders

e. Neurotic, stress-related and somatoform disorders
   
   (1) Reaction to severe stress
      
      • Acute stress disorder
      
      • Adjustment disorder
      
      • Post-traumatic disorder
   
   (2) Anxiety disorder
      
      • Generalized anxiety
      
      • Phobic anxiety
      
      • Panic disorder
   
   (3) Obsessive–compulsive disorder
      
      • Somatoform disorders
• Dissociate (conversion)disorder
• Neurasth

f. Behavioral syndromes associated with physiological disturbances

• Eating disorder
• Sexual dysfunction
• Sleep disorders
• Puerperal mutual disorders

g. Disorders in adult personality and behavior

• Personality disorder
• Factitious disorder

1.5.2. Anxiety

Anxiety Disorders affect about 40 million American adults age 18 years and older (about 18%) in a given year, causing them to be filled with fearfulness and uncertainty. Unlike the relatively mild, brief anxiety caused by a stressful event (such as speaking in public or a first date), anxiety disorders last at least 6 months and can get worse if they are not treated. Anxiety disorders commonly occur along with other mental or physical illnesses, including alcohol or substance abuse, which may mask anxiety symptoms or make them worse. In some cases, these other illnesses need to be treated before a person will respond to treatment for the anxiety disorder. Effective therapies for anxiety disorders are available, and research is uncovering new treatments that can help most people with anxiety disorders lead productive, fulfilling lives. Anxiety is a feeling of apprehension or fear, combined with symptoms of increased sympathetic activity, is a normal response to stress. A clinical problem may arise if the anxiety becomes severe or persistent, and interferes with everyday performance. Clinical subtypes of anxiety include panic disorder, agoraphobia, other phobias and generalized anxiety. The prevalence of such syndromes in the general population is about 10-20%, and there is a high rate of co-morbidity with depressive disorders (Judd LL et al., 1998). The overall female to male ratio is 2:1. The age of onset of most of the anxiety disorders is in young adulthood (twenties and thirties), although the maximum prevalence of generalized anxiety and agoraphobia-panic in the general population is in the 50-64 year age group.
1.5.2.1. Clinical categories of anxiety

- **Generalized Anxiety Disorder** is an ongoing state of excessive anxiety lacking any clear reason or focus. Essential feature of this class of anxiety is chronic worry (Gorman JM, 2003).

- **Panic Disorder** is an attack of overwhelming fear occurring in association with marked somatic symptoms such as sweating, unexpected recurrent panic attacks, tachycardia, chest pains, trembling, choking etc. normally this condition of anxiety has a general component (Tharmalingam S et al., 2006).

- **Post-traumatic Stress Disorder** elaborates an anxiety triggered by insistent recall of past 1 stressful experiences (Kathryn M et al., 2003).

- **Social Anxiety Disorder** is characterized by marked and persistent fear of performance situations when they feel, they will be the center of attention and will do something humiliating or embarrassing. Situation that provokes this fear may be quite specific e.g. public speaking (Lochner C et al., 2006).

- **Phobia** is a strong fear of specific things or situations e.g. snakes, open spaces, flying and social interactions (Iancu I et al., 2006).

Current pharmacotherapy of anxiety revolves around the use of synthetic molecules as well as drugs obtained from natural sources (plant origin). The role of Gamma amino butyric acidergic transmission is also been studied in detail because of its biological complexity and molecules that could specifically act at receptor subtypes are being explored that could mediate specific physiological effects while causing little side effects (Hardman JG et al., 2001). Stressful manipulations are known to alter gamma amino butyric acid (GABA) functions. It is not known as to what extent stress would affect the role of GABA in anxiety. Receptors within the glutamate, GABA and neuropeptide systems provide a rich diversity of drug targets in the treatment of anxiety (Maubach KA et al., 1999). Novel drugs specifically aim at new targets within these neurotransmitter systems as well as targets outside these systems (Kent JM et al., 2008). Increasing attention is being paid to post-synaptic regulation of nor-adrenergic receptors and alteration of the serotonergic neurotransmission in the treatment of depression and anxiety (Maubach KA et al., 1999). Serotonergic neurotransmission involves a rich diversity of receptor subtypes located either pre or post synaptically and are functionally different (Kent JM et al., 2008, Stahl SM 1998).

Drugs developed affect these targets would be advantageous in optimizing therapy.
These classes of molecules, originally introduced with the aim to benefit depressed patients are also clinically effective in the treatment of all anxiety disorders and are proving to be superior to benzodiazepines (Borsini Fi et al., 2002). However, the predictive validity of models of ‘anxiety’ remains to be ascertained for these classes of molecules (Bourin M et al., 1996). Three neurotransmitter systems are implicated in the biological basis of anxiety: The GABA-benzodiazepine receptor complex, the locus coeruleus norepinephrine system and serotonin. These neurotransmitters systems may mediate “normal” anxiety as well as pathological anxiety (Stahl SM 1998). However it is theorized that in case of anxiety, unlike depression, the noradrenergic system in the locus coeruleus region of the brain stem is overactive but does not account exclusively for its manifestation (Stahl SM 1998).

Benzodiazepine (BzD) receptors are unevenly distributed in the CNS and are found in the highest concentration in the hippocampus, striatum and spinal cord. Neurophysiological data shows that the limbic system is particularly sensitive to these receptor-mediated effects. Heterogeneity, density, functional states and activation of these BzD receptors are supposed to affect a variety of neuronal systems. In general, BzDs are supposed to lower the turnover of norepinephrine, dopamine, serotonin and acetylcholine (Hoehn-Saric R 1982). Most of the effects on BzD receptors are believed to be mediated via GABA\textsubscript{A} receptor-chloride channel complex (Hardman JG et al., 2001).

In general pharmacological manipulations that enhance serotonin also enhance anxiety, whereas serotonin may also reduce anxiety. 5-Hydroxy Tryptamine (5HT) is an inhibitory neurotransmitter; its origin is in the raphae nuclei and reticular system of the brain stem etc. they give rise to three major ascending pathways reaching the hypothalamus, the pre-optic and septal areas, the hippocampus and all cortical areas, and the corpus striatum. Studies indicate that serotonin is principal mediator of stress-induced anxiety effects and there is interaction between the serotonergic and noradrenergic (NA) system in the manifestation of anxiety (Kent JM et al., 2008). It is assumed that, as is the case with NA activity, blockade of excess of serotonin (presumed to be increased in neurons) could lead to anxiolytic effects (Stahl SM 1998). Eight different receptor subtypes regulate the 5-HT functions. Azapirone and buspirone are selective 5-HT\textsubscript{1A} partial agonists that are effective for Generalized anxiety disorder (GAD), but not for panic disorder. The 5-HT\textsubscript{1A} partial agonists reduce serotonergic
activity; 5HT reuptake inhibitors are effective in antipanic compounds (Dipiro et al., 1997). Efforts are on to find better molecules, which can offer selective advantage over the existing ones with no/minimal side effects. In this effort, drugs are being employed in two strategies, to dissect the functional and structural systems that operate in the normal CNS, thereby defining specificity of these drugs as well as the systems on which they act, and to provide means to develop appropriate drugs to correct pathophysiological events in the abnormal CNS (Bloom FE and Kupfer DJ 1995).

Drug treatment for anxiety disorders revolves around the concept of modulating the adrenergic and serotonergic pathways in the brain, which are postulated to be overactive. Alternately, drugs known to reduce the excessive activity of these neurotransmitters are employed in treating anxiety of these include $\alpha_2$-receptor (autoreceptor) agonist such as clonidine, which has inhibitory effect in shutting of excess norepinephrine secretion and $\beta$-receptor antagonists, which block excess actions of biologically synthesized norepinephrine (Stahl SM 1998). In general, molecules used in the treatment of anxiety include Selective Serotonin Reuptake Inhibitors (SSRIs) and benzodiazepines (BDZs). It has however been observed that chronically administered antidepressant drugs, particularly SSRIs, are clinically effective in the treatment of all anxiety disorders, while the clinical effectiveness of BzDs is limited to GAD or acute panic attacks (Borsini Fi et al., 2002). As a result, SSRIs are preferred over BzDs as first line pharmacotherapy of anxiety (Stein MB 2003).

These agents acutely increase serotonin concentrations at the synapse, resulting in greater activation of postsynaptic 5HT receptors. However, this also increases binding of 5HT to presynaptic 5HT$_{1A}$ autoreceptors, resulting in inhibition of further synthesis of serotonin (Stahl SM 1998). Hence a major drawback with this therapy is that chronic administration of SSRI is associated with decreased responsiveness of this autoreceptor. Therefore, Serotonin norepinephrine reuptake inhibitors (SNRIs) are also being prescribed to patients for improvement of this disorder (Stahl SM 1998). Despite the advent of new molecules in pharmacotherapy of anxiety, it is however, unfortunate that this disorder goes underrated, undiagnosed and untreated (Stahl SM 1998).

BzDs are most widely prescribed drugs as sedatives and anxiolytics. Although BzDs are clinically effective, adverse effects such as dependence, tolerance, withdrawal symptoms and cognitive impairment continue to hinder patient’s compliance. Since the underlying
pathologies of these disorders are not clearly known, it is difficult to predict which patient is likely to respond to a given medication (Katzung BG 2001). Consequently, the search for the new sedative-anxiolytics continues as has been focused on providing solutions to the deficiencies of existing medication. If the focus of research is towards safety and efficacy, looking for newer drugs from natural sources may be a ‘ray of hope’. Hence, our idea is to search for a better drug, from nature for the treatment of insomnia and anxiety. Herbal medications are becoming increasingly popular in the treatment of psychiatric disorders. Herbs like Valerian, Hops, Passion Flower, Lemon Balm, St. John’s Wart etc are used as sedatives and anxiolytics.

1.5.3. Epilepsy

Epilepsy is a major neurological disorder characterized by recurrent, spontaneous brain seizures or convulsions and its prevalence in developing countries is generally higher than in developed countries (Sander et al., 1996; Stafford et al., 2008). Epilepsy is the second most common neurological disorder after stroke and it is estimated that approximately 0.8% of the population is affected by some form of epilepsy (Pitkanen et al., 2009). Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose-related and chronic toxicity that involve virtually every organ system. Moreover, all the currently available AEDs have potential for adverse effects on cognition and behaviour (Duncan, 2002; Samren et al., 1997).

The practice of polypharmacy in the therapy of epilepsy that has doubtful background increases the risk of side effects and drug interactions. It can be said that all problems with the current AED therapy of epilepsy are more prevalent in underdeveloped countries due to lack of facilities for proper diagnosis, treatment and monitoring of serum levels of AEDs. Another critical issue associated with currently available AEDs is recent clinical and experimental data that strongly suggest that AED therapy does not alter the course or natural history of epilepsy and though AEDs suppress the seizures, they may not affect the underlying disorder (Chadwick, 1995; Loscher, 2002b; Shinnar et al., 1996). Only a very few AEDs have been shown to be antiepileptogenic including valproate and phenobarbitone (Duncan, 2002; Silver et al., 1991) and levetiracetam (Duncan, 2002; Loscher et al., 1998) but these are not well substantiated.

There is pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs which are not only anticonvulsant but also antiepileptogenic that
either prevent epilepsy or alter its natural course. Natural products and plants for that matter, used in traditional medicine can be an invaluable source for search for novel antiepileptic compounds (Meldrum, 1997; Stafford et al., 2008).

1.5.3.1. Pathophysiology of epilepsy

A variety of different electrical or chemical stimuli can easily give rise to a seizure in any normal brain. The epileptic seizure always reflects abnormal hypersynchronous electrical activity of neurones caused by an imbalance between excitation and inhibition in the brain. Neurones are interconnected in a complex network in which each individual neurone is linked through synapses with hundreds of others. A small electrical current is discharged by neurones to release neurotransmitters of synaptic levels to permit communication with each other. More than hundred neurotransmitters or neuromodulators have been shown to play a role in neuronal excitation. However, the major excitatory neurotransmitter in the brain is L-glutamate and the major inhibitory neurotransmitter in the brain is GABA.

An abnormal function of either of these could result in a seizure. A normal neurone discharges repetitively at a low baseline frequency, and it is the integrated electrical activity generated by the neurones of the superficial layers of the cortex that is recorded in a normal electroencephalogram. If neurones are damaged, injured or suffer electrical or metabolic insult, a change in the discharge pattern may develop. In the case of epilepsy, regular low-frequency discharges are replaced by bursts of high-frequency discharges usually followed by periods of inactivity. An epileptic seizure is triggered when a whole population of neurons discharges synchronously in an abnormal way. This abnormal discharge may remain localized or it may spread to adjacent areas, recruiting more neurons as it spreads.

1.5.3.2. Role of GABA and glutamate in the pathogenesis of epilepsy

It is important to emphasize the role of neurotransmitters especially, GABA and glutamate in epileptogenesis, since they are the major inhibitory and excitatory transmitters in the CNS, respectively, and the fact that generation of seizures has been attributed to imbalance between excitatory and inhibitory neurotransmission in epileptic brains. GABA plays an important role in regulation of neuronal excitability and impairment of GABA function produces seizures (Olsen et al., 1997). Compounds that
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Enhance GABA-mediated inhibition are convulsants (Scholze et al., 1996; Sieghart, 1992). GABA exerts its major inhibitory effect via GABA<sub>A</sub> receptor (which is a ligand-gated ion channel) by increasing neuronal membrane conductance for chloride ions causing membrane hyperpolarization resulting in reduced neuronal excitability and most rapid inhibition in brain (Sieghart, 1992). GABA<sub>A</sub> receptor is target for many important neuroactive drugs including antiepileptic drugs BzDs and barbiturates (Scholze et al., 1996; Sieghart, 1992). GABA<sub>A</sub> receptor consists of five subunits that form a chloride ion channel (Macdonald et al., 1994). The subunits consist of various subtypes and pharmacological studies have shown that individual subunits and subtypes confer different sensitivities to agents acting on GABA<sub>A</sub> receptors (Neelands et al., 1998). It is postulated that exposure of GABA to postsynaptic receptors for a brief period of time results in generation of Inhibitory Post-Synaptic Currents (IPSCs) (Hill et al., 1998).

GABA<sub>A</sub> receptor-mediated miniature IPSCs play important physiological role in preventing the development of neuronal hyper-excitability (Salin et al., 1996). Decrease in GABA<sub>A</sub> from receptor-mediated IPSCs is observed in cells from hippocampi of animals with chronic experimental epileptic seizures and humans with chronic intractable temporal lobe epilepsy (Isokawa, 1996).

Glutamate is the most important excitatory neurotransmitter in all rapidly conducting relay pathways of the motor and sensory systems of the outer tube of the CNS. It produces fast or prolonged synaptic excitation and triggers various calcium dependent processes in the target cells, including production of nitric oxide (Bienvenu et al., 2002). Glutamate is a transmitter in the corticospinal, corticostriatal pathways, intrahemispheric and interhemispheric association pathways, hippocampal circuits, primary afferents, and somatosensory and special sensory pathways, cerebellar afferents and excitatory interneurones. Glutamate acts via two types of receptors, ionotropic glutamate receptors (iGluR) which are ligand-gated cation specific channels and metabotropic glutamate receptors (mGluR) which are G-protein-coupled receptors. Ionotropic glutamate receptors are classified according to their prototype agonists: NMDA (N-methyl-D-aspartate), kainite and AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid). Practically all agonists are able to induce epileptic seizures and brain damage whereas antagonists have been shown to be anticonvulsant (Mares et al., 2004a; Mares et al., 2004b). The role played by metabotropic glutamate receptors depends on the type
of receptors: activation of type I is proconvulsant and convulsants, whereas activation of type II and III is anticonvulsant (Moldrich et al., 2003).

Epilepsy may arise as a consequence of a dramatic release of glutamate from central nerve terminals. Sustained seizures of the limbic system in experimental animals result in brain damage that resembles that due to glutamate toxicity. Similar changes are seen at autopsy in patients with intractable epilepsy. In animals such seizure-related brain damage may be reduced by the administration of non-competitive NMDA receptor antagonists, but it would appear that not all seizure activity is suppressed by drugs (Leonard, 2003).

The precise mechanism whereby persistent seizure activity results in neuronal degeneration is not completely understood. It seems possible that repetitive depolarization and repolarization of the nerve membrane eventually leads to an energy-deprived state within the cell, thereby preventing the restoration of the cell membrane potential. Each depolarization will also lead to an influx of calcium ions and efflux of potassium ions, which if prolonged, can result in cell death. The reduced efficiency of glial cells to remove potassium ions, and the ability of high extracellular concentration of potassium ions to depolarize neurons and cause neurodegenerative changes also play a critical role in causing the degenerative changes that are a feature of status epilepticus and intractable epilepsy (Leonard, 2003). Recent advances have indicated that GABA<sub>A</sub> receptors work synergistically with NMDA receptors to increase the influx of calcium ions into neuroblasts and immature neurons. This is essential for the modulation of early CNS development (DeLorey et al., 1999). It is evident that GABA is a critical inhibitory transmitter and seizures can rapidly be elicited by pharmacological disruption of GABAergic mechanism (Feldman et al., 1991). Drugs have also been developed to modulate glutamic acid function. Reduction of excitatory glutaminergic neurotransmission is potentially important; AMPA receptor blockade probably contributes to the antiepileptic effect of drugs such as lamotrigine (Lee et al., 2008).

1.5.3.3. Types of epilepsy

The clinical classification of epilepsy recognizes two categories, namely; partial seizures and generalized seizures, although there are some overlaps and many varieties of each. A seizure is said to be partial if it is restricted to a regional disturbance. Partial seizures are those in which the discharge begins locally and often remains localized. These may
produce relatively simple symptoms without loss of consciousnesses, such as involuntary muscle contractions, abnormal sensory experiences or autonomic discharge or they may cause more complex effects on consciousness, mood and behaviour, often termed psychomotor epilepsy (Rang et al., 2003). Generalized seizures involve the whole brain, including the reticular system, thus producing abnormal electrical activity throughout both hemispheres. Immediate loss of consciousnesses is characteristic of generalized seizures (Bienvenu et al., 2002).

The main categories are generalized tonic-clonic seizures (grand mal) and absences seizures (petit mal). A generalized tonic-clonic seizure consists of an initial strong contraction of the whole musculature, causing a rigid extensor spasm. Respiration stops and defaecation, micturition and salivation often occur. This tonic phase lasts for about 1 minute and is followed by a series of violent, synchronous jerks, which gradually dies out in 2-4 minutes. Most types of epilepsy are characterized by more than one type of seizure. Patients with focal (or partial) epilepsy may have simple partial, complex partial and secondarily generalized tonic-clonic seizures (e.g. partial seizures with secondary generalization). Patients with generalized epilepsy may have one or more of the following seizure types: absence, myoclonic, and tonic, clonic, tonic-clonic and atonic. Thus, no seizure type is specific for a single type of epilepsy. Seizures are symptoms, and patients should be treated for a type of epilepsy, not for a type of seizure (Benbadis et al., 2001).

1.5.3.4. Causes of epilepsy

Approximately 1% of the world’s population has epilepsy, the second most common neurological disorder after stroke (Porter and Meldrum, 2001). The cause of convulsions must be clearly understood through some precise observations. The type of seizure depends on the site of the focus in the brain. Epileptic attack can be caused by biochemical insults to the brain, such as hypoglycaemia, anoxia, hypocalcaemia, hyperventilation, water intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol (Bienvenu et al., 2002). Epilepsy can also be caused by previous 12 active pathology factors, such as birth trauma to the brain, during or following meningitis, trauma to the skull and brain later in life, cerebral abscesses, cerebral infarction, cerebral haemorrhage or subarachnoid haemorrhage (Bienvenu et al., 2002). Further analysis shows that the blockade of post-synaptic gamma-amino butyric acid
receptors or an inhibition of GABA synthesis is the principal origin of brain discharge. According to Bienvenu and co-workers (Bienvenu et al., 2002), an epileptic attack can be triggered by a sensory stimulus, which is specific for individuals. To date, there is no single unifying explanation as to how these diverse factors cause seizures. Hence, it is difficult to determine the exact cause of epilepsy, even though it has been possible to investigate the physiological events which participate in the genesis of epilepsy.

1.5.3.5. Mechanism of action of antiepileptic drugs

With the exception of valproate, the established AEDs tend to have clearly defined, single mechanism of which facilitates the prediction of effectiveness of treatment on the basis of pharmacology. At the cellular level, three major mechanisms of action of antiepileptic drugs are recognised; modulation of ion channels, enhancement of GABA inhibitory neurotransmission, and attenuation of glutamate mediated excitatory transmission (Kwan et al., 2001).

1.5.3.6. Modulation of ion channels

The intrinsic excitability of the nervous system is ultimately controlled by voltage-gated ion channels which regulate the flow of cations across surface and internal cell membranes. The sodium channel is arguably the most important and responsible for depolarization of the cell membranes and the characteristic upstroke of the neuronal action potential. Blockade of voltage-gated sodium channels is the most common mechanism of action amongst currently available AEDs (Deckers CL et al., 2003). Well established AEDs, phenytoin and carbamazepine are prototype sodium channel blockers and this mechanism is shared by the newer drugs lamotrigine, felbamate, topiramate and oxcarbazepine (Deckers CL et al., 2003). These drugs mainly bind to the inactivated state of the sodium channel and produce a voltage- and frequency-dependent reduction in channel conductance, resulting in a limitation of repetitive neuronal firing with little or no effect on the generation of single action potentials (Kwan et al., 2001). Voltage-gated calcium channels, likewise sodium channels, are involved in depolarization, often recruited in response to initial sodium-dependent action potential generation.

Calcium channels are distributed throughout the nervous system on dendrites, cell bodies and nerve terminals. The N-, P- and Q-type calcium channels have been implicated in the control of neurotransmitter release at the synapse, whereas the T-type channel, expressed
predominantly in the thalamocortical relay neurones, is believed to play a role in the distinctive rhythmic discharges of generalised absence seizures (Kwan et al., 2001). These channels represent a major target for AEDs. Ethosuximide efficacy against generalised absence seizures is believed to be mediated by blockade of the T-type calcium channel (Deckers CL et al., 2003). Evidence suggests that valproate may have similar effects (Deckers CL et al., 2003). Lamotrigine has also been reported to limit neurotransmitter release by blockade of the N- and P- subtypes of voltage-sensitive calcium channel while gabapentin binds to the α2δ-subunit of the L-type channel (Kwan et al., 2001).

1.5.3.7. Enhancement of inhibitory neurotransmission

GABA is the predominant inhibitory neurotransmitter in the mammalian CNS. Following synaptic release, GABA acts at three specific receptors, GABA_A, GABA_B, and GABA_C (Deckers CL et al., 2003). The GABA belongs to the ligand-gated ion channel superfamily and responds to GABA binding by increasing chloride conductance, resulting in neuronal hyperpolarization. GABA is removed from the synaptic cleft into localised nerve terminals and glial cells by specific transport molecules. Thereafter, GABA is either recycled to the readily releasable neurotransmitter pool or metabolized by the action of the mitochondrial enzyme GABA-transaminase, thereby completing the cycle (Kwan et al., 2001).

Phenobarbital and the benzodiazepines bind to distinct sites on the GABA_A receptor complex and exert an allosteric influence on the opening of the chloride ion channel in response to GABA. Phenobarbital increases the duration of channel opening, while the benzodiazepines increase the frequency of opening (Deckers CL et al., 2003). Vigabatrin and tiagabin exert their antiepileptic actions by selective effects at the GABA synapse. Vigabatrin is an irreversible inhibitor of the enzyme GABA-transaminase, while tiagabin prevents the uptake of GABA from the synaptic cleft by blockade of the GAT-1 transporter.

1.5.3.8. Attenuation of excitatory neurotransmission

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following synaptic release, it exerts its effects on both ionotropic and metabotropic receptor types. The ionotropic glutamate receptors are arguably the best characterized
and are classified into three subtypes, AMPA, kainite and NMDA, which form ligand-gated ion channels permeable to sodium and depending on subtype and subunit composition, calcium ions. The AMPA and kainite subtypes are implicated in fast excitatory neurotransmission, whereas the NMDA receptor, quiescent at resting membrane potential, is recruited during periods of prolonged depolarization (Kwan et al., 2001). None of the current AEDs available exerts its pharmacological effects solely by an action on the glutamatergic system (Deckers CL et al., 2003). However, blockade of the NMDA subtype of glutamate receptor has been reported to contribute to the antiepileptic effects of felbamate (Deckers CL et al., 2003). Topiramate is similarly distinguished by an inhibitory action on AMPA receptors. Furthermore, several AEDs have been reported to reduce glutamate release, although this effect may be more indicative of their actions on calcium channels than a direct effect on the glutamate system (Kwan et al., 2001).

1.5.4. Alzheimer disease

Alzheimer’s disease is a primary degenerative disease of the brain, characterized by progressive memory impairment. AD was originally recognised by Alois Alzheimer in 1907 as a form of dementia. Alzheimer’s dementia is the most common form of cognitive impairment in older persons. It afflicts about 5–10% of those in the community over the age of 65 and almost half of those over the age of 85 (Reiman EM et al., 1999). Since the population continues to grow older, AD is expected to take an increasing toll on those affected, their families, and the communities in which they live. In the last few years researchers have made significant studies in the discovery of genetic and non genetic risk factors for AD, molecular event’s which might lead to AD disease neuropathology, and interventions which possibly decrease the risk, delay the onset, or slow the progression of this devastating disorder (Kelley BJ et al., 2007).

A patient with AD will generally present a gradual onset, progressive and sequential decline in cognitive, behavioral and motor functions. This decline will eventually interfere with the individual’s daily functioning and quality of life.

- Cognitive symptoms include loss of short-term memory, language impairment, disorientation to time, place and people.
- In the early stages, patients may exhibit symptoms of depression.
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❖ At the later stages, behavioral and psychiatric symptoms such as agitation, aggressivity, delusions, and hallucinations may develop.
❖ Finally, in the more advanced stages, motor functions decline and patients may become incontinent and, at times, bedridden.

1.5.4.1. Prevalence of Alzheimer’s disease

The prevalence of dementia related to AD, after the age of 65 years is doubling for every 5.1 year age interval. The prevalence of dementia in the general population over 65 years is estimated to vary between 2.2 and 8.4% with AD accounting for approximately 45–67% of all forms of dementia. Alzheimer’s Disease International’s (ADI) report provided up-to-date information on the prevalence and impact of dementia from a global perspective (Wimo A et al., 2010). The report estimated that 35.6 million people living with AD dementias worldwide in 2010, increasing to 65.7 million by 2030 and 115.4 million by 2050. The report of ADI highlighted that nearly two-thirds of all people with dementia lived in low and middle income countries, this proportion being set to grow because the sharpest increases in the numbers of people with dementia will be in rapidly developing regions including Latin America, China and India.

1.5.4.2. Etiology and genetics

The exact etiology of AD is unknown; however, several genetic and environmental causes have been explored as potential causes of AD. Almost all early onset cases of AD can be attributed to alterations on chromosomes -1, 14, or 21. The majority and most aggressive early onset cases are attributed to mutations of a gene located on chromosome 14, which produces a protein called presenilin-1. A structurally similar protein presenilin-2, is produced by a gene on chromosome-1. Both presenilin-1 and presenilin-2 encode for membrane proteins that may be involved in amyloid precursor protein (APP) processing. Scientists have identified more than 160 mutations in presenilin genes, and these mutations appear to result in reduced activity of γ-secretase, an enzyme important in β-amyloid peptide (βAP) formation. APP is encoded on chromosome-21. Only a small number of early onset familial AD cases have been associated with mutations in the APP gene, resulting in overproduction of βAP or an increase in the proportion of βAP ending at residue 42 (Findeis MA, 2007).

Genetic susceptibility to sporadic, late-onset AD is thought to be primarily linked to the apolipoprotein E (apo-E) genotype. Thus far, the contribution of other candidate genes
appears to be minor, although AD may be a heterogeneous disease resulting from complex interactions among multiple susceptibility genes and environmental factors. The gene responsible for the production of apo E is located on chromosome-19. There are three major subtypes or alleles of apo E (e.g., apo-E2, apo-E3, and apo-E4). Humans inherit one copy of the apo-E gene from each parent. Inheritance of the apo-E4 allele is believed to account for much of the genetic risk in sporadic AD. The mechanism through which apo-E4 confers an increased risk is unknown. Although apo-E4 is associated with other factors that may contribute to AD pathology, such as abnormalities in mitochondria, cytoskeletal dysfunction, and low glucose usage. The degree of risk depends on such factors as the number of apo-E4 copies, age, ethnicity, and gender. Overall, approximately 40% of patients with late-onset AD have at least one copy of apo-E4. Individuals homozygous for apo-E4 are at increased risk, and as many as 90% of persons inheriting two copies of apo-E4 will develop AD by age 80 years. Moreover, onset of symptoms occurs at a relatively younger age as compared to patients having zero or only one copy of apo-E4 in their genotype. In whites, inheriting a single copy of apo-E4 increases AD risk, whereas inheriting the apo-E2 allele may protect against AD. Although inheritance of the apo-E4 allele increases the risk of AD, it is not diagnostic or even essential for disease presence.

Apo-E4 is the only genetic factor that is unequivocally associated with an increased risk of late-onset AD, but it has been estimated to account for less than half of the genetic contribution to AD risk. This polymorphism may also be more prevalent in the white population. Because the incidence of AD is higher in other populations, AD occurs at an early age in some individuals, and AD is associated with vascular risk factors such as obesity, diabetes, and hypertension. Genetic explanatory factors continue to be investigated. Genetic factors have been linked to both early and late-onset AD. Alterations to chromosomes 1, 14, and 21 are associated with early onset AD, whereas the presence of apo-E4 alleles increases risk of developing late-onset AD (Pharmacotherapy, 2008).

1.5.4.3. Pathophysiology

The signature lesions in AD are neuritic plaques and neurofibrillary tangles (NFTs) located in the cortical areas and medial temporal lobe structures of the brain. Along with these lesions, degeneration of neurons and synapses, as well as cortical atrophy, occurs.
Plaques and NFTs may also be present in other diseases, even in normal aging, but there is a much higher concentration of plaques and NFTs in patients with AD (Maccioni RB et al., 2001). The circumstances in which these lesions lead the clinical picture of AD remain unclear. Several mechanisms have been proposed to explain these changes in the brain, including Aβ aggregation and deposition leading to the formation of plaques; hyperphosphorylation of tau protein leading to NFT development; inflammatory processes; dysfunction of the neurovasculature; oxidative stress; and mitochondrial dysfunction.

1.5.4.4. Amyloid Cascade Hypothesis

Amyloid-β (Aβ), a 40- to 42-amino-acid peptide, is derived by proteolytic cleavage of an integral membrane protein known as APP by the action of β- and γ-secretases. Aβ constitute the majority of the plaques found in human brain and have been considered to play a role in the development and progression. A number of studies suggest that small oligomers of Aβ are the actual toxic species of this peptide, rather than Aβ fibrils (Edelberg HK et al., 1996; Newman M et al., 2007). In addition, genetic mutations in genes for APP or presenilin-1 or presenilin-2, which lead to familial AD, have been reported to increase the production of Aβ and lead to AD (Morgan C et al., 2004).

![Figure 3. Formation of Amyloid plaques.](image)

1.5.4.5. Neurofibrillary Tangles

NFTs are commonly found in the cells of the hippocampus and cerebral cortex in persons with AD and are composed of abnormally hyperphosphorylated tau protein. Tau protein provides structural support to microtubules, the cell’s transportation and skeletal support system. When tau filaments undergo abnormal phosphorylation at a specific site, they cannot bind effectively to microtubules, and the microtubules collapse. Without an intact system of microtubules, the cell cannot function properly and eventually dies.
(Pharmacotherapy, 2008; Vickers JC et al., 2000). The density of the NFTs correlates well with the severity of the dementia, because they are a hallmark of neuronal death. NFTs are found in other dementing illnesses besides AD, and may represent a common method by which various inciting factors culminate in cell death.

Figure 4. Shows the process of NFTs formation

1.5.4.6. Inflammation In AD

The inflammatory hypothesis claims that amyloid excess may have direct neurotoxicity at least some of its toxicity might actually be an indirect consequence of a βAP protofibril induced microglia activation and astrocyte recruitment. This inflammatory response may represent an attempt to clear amyloid deposition. However, it is also associated with release of cytokines, nitric oxide, and other radical species, and complement factors that can both injure neurons and promote ongoing inflammation. Indeed, levels of multiple cytokines and chemokines are elevated in AD brains, and certain proinflammatory gene polymorphisms are reported to be associated with AD (Heneka MT, 2006). Like other inflammatory diseases, AD is associated with the upregulation of a diverse set of acute phase proteins that arise early in inflammation. Acute phase mechanisms involved in the initiation, clearance and subsequent tissue rebuilding process after injury are coordinated by the pleiotrophic actions of numerous molecules. Acute phase molecules that signal the pro-inflammatory mechanisms of wound healing include interleukin-1 (IL-1), IL-6, TNFα, intracellular adhesion molecules (ICAM), colony stimulating factors (CSF) and acute phase proteins such as C-reactive protein.
1.5.4.7. The cholinergic hypothesis

Multiple neuronal pathways are destroyed in AD. Damage occurs in any nerve cell population located in or traveling through plaque laden areas. Widespread cell destruction results in a variety of neurotransmitter deficits, with cholinergic abnormalities being the most prominent. Loss of cholinergic activity correlates with AD severity. In late AD, the number of cholinergic neurons is reduced, and there is loss of nicotinic receptors in the hippocampus and cortex. Presynaptic nicotinic receptors control the release of acetylcholine, as well as other neurotransmitters important for memory and mood, including glutamate, serotonin, and norepinephrine (Pharmacotherapy, 2008). The discovery of vast cholinergic cell loss led to the development of a cholinergic hypothesis linked to the pathophysiology of AD. The cholinergic hypothesis targeted cholinergic cell loss as the source of memory and cognitive impairment in AD. Consequently, it was presumed that increasing cholinergic function would improve symptoms of memory loss. This approach is flawed for two reasons. First, cholinergic cell loss appears to be a secondary consequence of Alzheimer’s pathology, not the disease-producing event; second, cholinergic neurons are only one of many neuronal pathways destroyed in AD. It is increasingly clear that simple addition of acetylcholine cannot compensate for the loss of neurons, receptors, and other neurotransmitters lost during the course of the illness. Thus the goal is to minimize or improve symptoms through augmentation of neurotransmission at remaining synapses.

1.5.4.8. Oxidative stress in AD

Within any functional, aerobic cell, the processes involved in respiration inevitably generate reactive oxygen species (ROS). In particular, the oxidation reduction reactions necessary for the generation of ATP (via the establishment of a proton gradient in oxidative phosphorylation) produce free radical intermediates as electrons are transferred from one molecule to another (Behl C, 1998). Despite the resident sequestration mechanisms present within the cell that prevent the potentially harmful dispersion of the free radical intermediates, a substantial amount of ROS manage to escape daily, free to wreak havoc on macromolecules. In fact, in a specialized cell with high metabolic activity, such as a neuron, the number of such free radicals produced is estimated by some to be 1011 ROS/cell/day (Chauhan V et al., 2006). This damaging effect is most notable in AD. That is, oxidative damage marked by lipid peroxidation, nitration,
reactive carboxyls. Specifically, cytochrome oxidase, the pyruvate dehydrogenase complex, and the ketoglutarate dehydrogenase complex show reduced activity as a result of oxidative damage and nucleic acid oxidation is increased in vulnerable neurons in AD relative to unaffected patients, whether or not they contain any other corresponding pathology (i.e. NFTs). Furthermore, reduced metabolic activity, deemed the result of oxidative damage to vital mitochondrial components, has been demonstrated in AD. Compared to all other tissues, which can potentially be damaged by ROS, the brain is particularly vulnerable to oxidative processes for several reasons:

1. Neurons of the CNS are almost completely dependent on the oxidative phosphorylation reactions in order to generate adenosine triphosphate as energy source.
2. For the normal adult brain, glucose is the major nutrient and, therefore, the brain has a high glucose metabolism and respiratory turnover.
3. Neuronal membranes of the brain consist of high concentrations of polyunsaturated fatty acids, which are potential substrates for the peroxidation by hydroxyl radicals.
4. The brain has an overall high concentration of catalytic iron.
5. The brain has only low levels of antioxidant defense enzymes compared to other tissues.

1.5.4.9. Sources of ROS in the brain

In addition to the oxidative phosphorylation in the mitochondria, numerous other enzymatic and non-enzymatic cellular mechanisms exist that can generate O$_2^-$ or H$_2$O$_2$. Moreover, the production of ROS can result from the enzymatic conversion of catecholamines and indolamines by monoamine oxidase or from the non-enzymatic auto-oxidation of catecholamines. Superoxide can be produced from lipoxygenases, cyclooxygenases (COX) and various oxidases. As the sources of ROS in nerve cells are numerous, the cells have to maintain an effective antioxidant defense system in order to protect themselves against free radical damage (Smith MA et al., 2000). Enzymatic and non-enzymatic antioxidants keep the balance between the physiological production of ROS and their detoxification. Because the oxidative phosphorylation, the main physiological source of ROS, is located within the microenvironment of the mitochondria, the free radicals are quickly reduced to H$_2$O. Leaking O$_2$ can be dismutated by super oxide dismutase (SOD) to H$_2$O$_2$. Non-enzymatic and so-called
chain-breaking antioxidants, such as the lipophilic free radical scavenger \(\alpha\)-tocopherol (vitamin-E) and the hydrophillic ascorbate (vitamin C), the two most prominent antioxidants of their class, can directly react with ROS at the molecular level. In addition to its direct reaction with ROS, ascorbate is also necessary to regenerate vitamin-E. An increased production of free radicals either induced by an overdrive of endogeneous ROS generating systems or by exogenous oxidative insults challenges this intracellular balance maintained by the various antioxidants, ultimately, leading to a state of oxidative stress with massive cell damage and cell death.

1.5.4.10. Diagnosis

Diagnosis of AD is problematic for there is no specific biological marker for the disease. In practice, patients are screened and are then diagnosed for AD based on a physical exam, patient history and a systematic determination of their mental state using specific cognitive and psychological tests. The screening tool most often encountered and used in epidemiological and clinical studies to assess overall mental status is the mini-mental state examination (MMSE), or the modified mini-mental state examination (3MS). Another instrument, mostly encountered in prevalence studies, is the Cambridge mental disorders of the elderly examination (CAMDEX), which includes the MMSE, as well as the Blessed dementia rating Scale \((\text{Pharmacotherapy, 2008})\).

Scanning techniques also provide useful indication in the diagnosis of AD. Computerized tomography (CT), Magnetic resonance imaging (MRI) and Positron emitting tomography scan (PET Scan) are used clinically to evaluate changes in brain of the patient suffering from AD. Serologic evaluation includes blood cell counts, serum electrolytes, liver function tests, a test of thyroid function, and a vitamin \(B_{12}\) level.

1.5.4.11. Current approaches in the treatment of AD

The proposed pathogenic mechanisms for AD generally comprise the basis for current attempts at therapeutic intervention \((\text{Farlow MR et al., 2007})\). These include loss of cholinergic function (cholinergic replacement therapy and neurotropins), oxidative stress (antioxidant therapy), the amyloid cascade (A\(\beta\) vaccine, band g-secretase effectors, statins), inflammatory mediators non steroidal anti-inflammatory drugs (NSAIDs), steroid hormone deficiencies (hormone replacement therapy), excitotoxicity (memantine), and the role of dietary factors (low saturated fat diets, moderate alcohol intake).
1.5.4.12. Acetyl cholinesterase (AChE) inhibitors

The clinical use of acetylcholinesterase inhibitors for the treatment of AD began in the early 1980s with the administration of physostigmine by several investigators. The results of these trials were encouraging from the standpoint that cognition could be improved using this approach. Unfortunately, the clinical potential of physostigmine was limited by its short duration of action (half life of 30 min), which required frequent administration, its narrow therapeutic window, and its high incidence of side effects (e.g., sweating, muscle fasciculations, cramps, nausea, vomiting). A partial breakthrough came about with the recognition that the oral administration of tacrine might be beneficial in AD Tacrine, an aminoacridine, is a reversible inhibitor of AChE (Small GW, 1998). Like physostigmine, tacrine is reversible in the sense that it binds only weakly to the active site of AChE, resulting in a relatively short half-life of enzyme inhibition. Several large clinical trials subsequently confirmed the beneficial effect of tacrine on cognition in AD. Although tacrine offers some advantages over physostigmine, including a somewhat longer half-life (2 to 4 hrs), it still requires dosing four times a day.

Additionally, tacrine has a relatively narrow therapeutic window, with its greatest therapeutic effect occurring at doses that are frequently associated with cholinergic side effects. At doses of 80 to 160 mg, tacrine is associated with nausea and vomiting in 35% of patients.

Donepezil was the second AChE inhibitor to be approved by the Food and drug administration (FDA) for the treatment of AD. It is currently the most commonly prescribed acetylcholinesterase inhibitor for the treatment of AD. Donepezil is a piperidine and, like tacrine, is a reversible acetylcholinesterase inhibitor. It has several advantages over tacrine. It can be administered only once per day because it is extensively bound to plasma proteins and has a plasma half-life of approximately 70 hrs.

1.5.4.13. Antiglutamatergic therapy

Memantine is the only N-methyl-D-aspartate (NMDA) antagonist currently available. Memantine blocks glutamatergic neurotransmission by antagonizing NMDA receptors. Glutamate is an excitatory neurotransmitter in the brain implicated in long-term potentiation, a neuronal mechanism important for learning and memory. Glutamate is the
principle excitatory neurotransmitter incortical and hippocampal neurons. Glutamine synthetase is oxidized in the brains of individuals with AD, leading to excess glutamate. Excessive activation of NMDA receptors by glutamate increases the vulnerability of CNS neurons leading to neuronal degeneration. Memantine blocks glutamate gated NMDA channels, thereby blocking pathological activation and preserving physiological activation. By blocking NMDA receptors, excitotoxic reactions, which ultimately lead to cell death, may be prevented (Pharmacotherapy, 2008).

1.5.4.14. Non-Steroidal Anti-inflammatory Drugs

Aβ deposition and plaque formation are associated with an innate immune response that includes activation of complement, secretion of pro-inflammatory cytokines, expression of chemokines, and excretion of nitric oxide which mediates apoptosis NSAIDs downregulate pro-inflammatory signals, microglia, and astrocytes and may reduce risk of AD by lowering Aβ production. The role of selective and non selective cyclooxygenase (COX) inhibitors in AD is becoming clear as the research on these agents proceeds on day by day (Hirohata et al., 2005). Various agents like nimesulide, ibuprofen, indomethacin and acetaminophen appear to have protective effect in the AD (Aisen PS, 2002). These agents prevent the brain against the inflammatory damage by reducing the extent of inflammation and preventing the accumulation of various inflammatory proteins at the site of damage.

1.5.4.15. Antioxidants and steroidal hormone therapy

Various studies had established a relation between AD and oxidative stress so treatment with antioxidants like vitamin-E is becoming a vital player of the therapy (Behl C et al., 2002). Other agents like vitamin C and carotenoids like beta carotene etc are used as a antioxidant nutraceutical.

Estrogen enhances cerebral blood flow, prevents atrophy of cholinergic neurons, reduces oxidative stress, and modulates the effects of nerve growth factor. It may also reduce neuronal injury by decreasing formation of Aβ. Three prospective, population based epidemiologic studies suggested that postmenopausal estrogen replacement therapy (ERT) may delay the onset of AD. Estrogens may improve memory and aspects of other cognitive performance through a variety of mechanisms, including cholinergic neuroprotective and neurotrophic effects. In studies of ovariectomized rats, estradiol replacement enhanced learning and increased neuronal choline uptake and choline acetyltransferase (Pharmacotherapy, 2008).


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**Chapter – I**

**Introductions**


