According to world health organization (WHO) “drug dependence” means a strong craving to take the addictive substance or drug of abuse. As per the guidelines of Diagnostic and Statistical Manual of Mental Disorders following features must be experienced by the subject:

- Strong craving or urge to take the drug of abuse,
- Recalcitrant behaviour to take addictive substance or drug,
- Continuous use of the drug of abuse to attenuates the withdrawal syndrome,
- Continuous and prolong use of drug to achieve desired result due to tolerance,
- No interest in other pleasurable activities and chronic use of drug despite their harmful effects.

Nicotine dependence is characterized by both tolerance and withdrawal symptoms in relation to nicotine use. Nicotine dependence can occur with cigarette smoking, smokeless tobacco use, cigar or pipe use (World Health Organization, 2003).

Physical dependence is characterized by desensitization to elements of nicotine intoxication (tolerance), sensitization to nicotine-induced incentive salience (craving), and withdrawal after pharmacokinetic elimination. As stated in DSMIV-TR, withdrawal symptoms include depressed mood, insomnia, irritability, anxiety, difficulty concentrating, restlessness, and weight gain or increased appetite. The repetitive use of nicotine, compelled by positive reinforcement (pursuit of pleasure) and negative reinforcement (avoidance of withdrawal), also becomes a psychologically conditioned behavior within time moods, environments, behaviors, and sensations become conditioned cues that independently trigger the craving to smoke (Ziedonis and George, 1997; Danovitch, 2011).
2.1 NICOTINE DEPENDENCE:

Nicotine is a naturally occurring alkaloid found mainly in the members of the solanaceous plant family such as potato, tomato, green pepper and tobacco. Major preponderance psychological and physiological effects in addicts are produced by levorotatory isomer of nicotine, which is a tertiary amine in nature and constitutes main component of tobacco smoke. Nicotine can easily cross blood brain barrier and cell membrane at physiological pH due to its basic nature. Nicotine acts as an agonist for nicotinic acetylcholine receptors (nAChRs) present in central nervous system and peripheral nervous system (Benowitz, 1988). Nicotine activates sympathetic nervous system that would lead to increased level of catecholamine which causes reduction in body weight and increased blood pressure. Neuronal nicotinic acetylcholine receptors are ligand-gated ion channels that are located in presynaptic terminals, somato dendritic, axonal and postsynaptic sites. Administration of nicotine modulates release various excitatory and inhibitory neurotransmitters throughout the brain (McGehee & Role, 1995; Wonnacott, 1997). nAChRs comprises five membrane-straddling subunits that combine to make a functional receptor (Changeux and Taly, 2008) that includes three isoforms of the neuronal β-subunit (β2–β4) and nine isoforms of the neuronal α-subunit (α2–α10). These subunits combine with a stoichiometry of two α- and three β-, or five α7-subunits to form nAChRs with distinct pharmacological and pharmacokinetic properties. Acetylcholine acts as an endogenous neurotransmitter that binds and activates nicotinic acetylcholine receptors at presynaptic terminals (Wonnacott, 1997). Nevertheless, nAChRs also are located at somato dendritic, axon and postsynaptic sites. Nicotine addiction is a complex behavioral phenomenon dependent on several systems, but the main reinforcing effect of nicotine depends on the activation of the mesolimbic dopaminergic system. Infusion of the nicotine antagonist into the cerebral ventricles or lesions of the mesolimbic dopamine neurons abolishes both locomotor activating and rewarding effects of nicotine (Portugal and Gould, 2007). Due to the extensive distribution of nicotinic acetylcholine receptors in CNS, administration of nicotine or nicotinic receptor agonists stimulates the release of various neurotransmitters like dopamine, epinephrine, glutamate etc. throughout the brain (McGehee and Role,
1995) which are involved in the rewarding effects of nicotine. These behavioral abnormalities develop gradually and progressively during a course of repeated exposure to a drug of abuse, and can persist for months or years after discontinuation of drug use. As a result, drug addiction can be considered a form of drug-induced neural plasticity (Nestler, 1993). The stability of the behavioral abnormalities that define addiction suggests a role in gene expression in this process (Nestler et al., 2001). According to this view repeated exposure to a drug of abuse alters the amounts, and even the types, of genes expressed in specific brain regions. Such altered expression of genes, then mediates altered function of individual neurons and the larger neural circuits within which the neurons operate. Ultimately, such neural circuit changes underlie the behavioral abnormalities seen in drug addicts (Nestler et al., 1993; Hyman and Malenka, 2001; Nestler et al., 2001a). There are many mechanisms by which repeated exposure to a drug of abuse could alter gene expression in the brain. These include altered rates of transcription of genes, altered processing of primary RNA transcripts into mature mRNAs, altered translation of these mRNAs into proteins, altered processing of proteins, and altered trafficking of mature proteins to their intracellular sites of action (Nestler et al., 2001a). Of all these mechanisms, the best understood, and the one which has received most study to date, is the regulation of gene transcription. Drug perturbation of synaptic transmission causes changes in numerous intracellular signaling pathways, which eventually signal to the cell nucleus, where specific proteins, called transcription factors, are altered. Transcription factors bind to short sequences of DNA located in the regulatory regions of genes and thereby control the rate of gene transcription. Over the past decade, drugs of abuse have been shown to alter many types of transcription factors in a variety of brain regions (O’Donovan et al., 1999; Berke and Hyman, 2000; Nestler et al., 2001a; Mackler et al., 2003).

Therefore, Nicotine dependence merits an exhaustive investigation to identify various related and other novel pharmacological approaches to ameliorate the pathological condition of nicotine dependence.
2.2 EPIDEMIOLOGY OF NICOTINE DEPENDENCE:

As per the estimates of world health organization there are more than 1.5 billion smokers throughout world in this day and age, which contributes about more than 5 million deaths each year means 1 in 10 adult deaths. Due to continuous smoking, men lost average age of 13.2 years whereas women smokers lost about 14.5 yrs (Das et al., 2012). It is estimated that 1/3 of the world’s adult population smokes tobacco. Tobacco is the leading cause of premature death in developed countries; those who smoke in early age will die from smoking related disorders like cardio vascular diseases, cancer and chronic obstructive pulmonary disease (COPD). In developing countries use of tobacco products and cigarette smoking is on rise, thus it becomes one of the major reasons of mortality in worldwide. In 2015, ten percent of the global deaths are linked with tobacco smoking.

2.3 WITHDRAWAL SYMPTOMS IN HUMAN:

Discontinuation and abruption of tobacco intake via cigarette smoking or any other source precipitates affective and somatic withdrawal symptoms, which includes palpitation, decreased heart rate, anxiety, rigorous craving for nicotine, loss of attentiveness, impatience, depressed mood, restlessness, weight gain and increased appetite (Corwin et al., 2006). These nicotine induced withdrawal syndrome generate anxiety and discourage persons taking large amount of nicotine to practice restraining oneself from indulging in nicotine which produces drive to relapse. Anxiety and stress play a complex role in nicotine dependence and withdrawal because nicotine addicts use cigarettes smoking as a means to diminish stress and anxiety to maintain the calming effect of cigarette, anxiety increases during the withdrawal. Several studies have shown that smokers continue to smoke to avoid the deprivation symptoms of nicotine. Besides being the symptom of withdrawal, anxiety and stress have the ability to worsen its symptoms, which result in increased craving and relapse due to nicotine dependence. Studies have also shown that subject with a history of depression are more sensitive to the effects of nicotine and show increased craving and withdrawal score on nicotine induction. Another study has shown that post traumatic stress disorder (PTSD) is connected with amplified cigarette smoking.
behavior and increased nicotine withdrawal symptoms and appear in mood regulation, which is one of the factors for promoting cigarette and preventing cigarette (Dani et al., 2011).

2.4 WITHDRAWAL SYMPTOMS IN RODENTS:

$\alpha_5$, $\alpha_3$, and $\beta_4$ subunits of nAChRs play a major role in nicotine dependence induced withdrawal syndrome by regulating release of various neurotransmitter. The VTA/NAc and the dopaminergic system are established in reinforcement of rewarding behaviors, and the interpeduncular nucleus and medial habenula axis is rising as the important anatomical structures responsible for the aversive and withdrawal effects of nicotine in rodents (Dani et al., 2011). Withdrawal symptoms can be observed in animal by discontinuing sudden chronic administration of nicotine thus, withdrawal symptoms can be recorded by observations of behavior signs or by disruption in operant behavior. Withdrawal can be seen in nicotine administrated rodent by systemically administering nAChR antagonists. Withdrawal symptoms can be somatic (physical) and affective (non-somatic) or both.

2.4.1 Somatic signs:

Studies show somatic signs that, as first documented in rats by chronic administration of nicotine and include symptoms like wet dog shaking, tremors, chews, teeth chattering, piloerection, gasps, shakes, yawns and palpebral ptosis these signs depend upon the amount of nicotine intake and can be overcome after treatment. Another method to produce withdrawal symptoms as shown by different studies is by chronic administration of nicotine and then injecting nicotine antagonist like mecamylamine systemically, which have slightly higher affinity for $\alpha_3\beta_4$ nAChRs than for $\beta_2$ receptors (De Biasi and Dani, 2011). Mecamylamine is the most excellent antagonist for precipitating nicotine dependence linked withdrawal signs. Genetic engineering technique have shown the study of nicotine withdrawal in mouse here, the symptoms of withdrawal are qualitatively similar to rat, but a sharp increase in certain normal behavior
becomes repetitive and more frequent which include shaking, grooming, scratching and also include jumping (De Biasi and Salas, 2008).

2.4.2 Non-Somatic signs:

Conditioned fear, anxiety like behaviour, conditioned place aversion and anhedonia are four most basic effective sign of nicotine dependence induced withdrawal signs. Major symptoms seen in nicotine withdrawal are diminished interest in rewarding stimuli, anhedonia, depression also produces anhedonia, is measured in rats as an increase in brain-stimulation reward thresholds (De Biasi and Salas, 2008).

2.5 PHARMACOTHERAPIES OF NICOTINE DEPENDENCE:

Treatment of nicotine dependence includes:

1. Pharmacological measures:

2. Non-pharmacological measures:

2.5.1 PHARMACOLOGICAL MEASURES INCLUDES:

The drugs currently approved by the United States of Food and Drug administration for smoking cessation include:

i. Nicotine replacement therapy (NRT):

It acts by relief of craving and withdrawal symptoms when a person stops tobacco use. It has reduced receptor responsiveness to acetylcholine, which bring satisfying factor when cigarette is taken by a person (Benowitz, 2008). NRT may be done with or without additional counseling and does not require to be prescribed by doctors. People using NRT during a quit attempt are likely to have a greater chance of success rate by using the combination nicotine patch and faster acting form (Stead et al., 2008).
ii. NICOTINIC AGENTS:

a. Transdermal nicotine patches: These are applied through the skin at steady state. Four patch formulations are present in the market currently are NicoDerm, CQ patch, Nicotrol patch, Habitrol patch, they vary in design, pharmacokinetics and duration of wear (Kumar et al., 2011). One of the advantages of these patches is that their continuous use in the treatment of nicotine dependence also benefits the characteristic lapse (Ferguson et al., 2012).

b. Gum: It is available to the subjects as transmucosal delivered nicotine polacrilex (nicotine gum), It is available in two doses strength of 2 mg and 4 mg respectively, that delivers 1 mg and 2 mg of nicotine, correspondingly. As per the instructions for using these gums one should use a part of gum for maximum two hours for the first six weeks, than reduce it to four hours for next three weeks than for next three weeks one piece of gum used for four to eight hours (Kumar et al., 2011). Prolonged use of gum causes gum disease as nicotine constricts blood vessels of gum (Christen et al., 1985).

c. Lozenges: The mechanism of action is that it is absorbed slowly through buccal mucosa and then circulated in the blood. It should not be chewed (Kumar et al., 2011). Studies show that smokers who have taken the treatment before with other drugs have good outcomes with active lozenges treatment (Shiffman et al., 2004).

d. Nicotine inhalers: It consists of a mouth piece and a plastic cartridge containing 10 mg of nicotine, out of which 4 mg can be delivered for systemic effect and 2 mg is captivated (Das et al., 2012). Pulmonary nicotine delivery in nicotine inhalers can be maximized by use of nicotine salt which has more physiological pH than pure nicotine where mass of the particle should be optimal for alveolar absorption. Flavoring agents can be added to enhance subject compliance (Caldwell et al., 2012).

e. Vaporizer: Vaporizer is a device mainly used to discharge the active ingredients of cannabis or tobacco (commonly used plant material), rather than flaming the plant herb, it heats the material, ideally to 180°C (356°F), due to
which the active compounds convert into aromatic vapour that contains no particulate material like tar and decrease harmful carbon monoxide gases. Inline water or ice attachments are used in water pipe to filter and cool the vapours and then inhaled straight, with pipe, or preserve consequent inhalations in a dome or balloon shaped container. Modest smoke is produced at low temperatures, through this system; one can achieve the same smoking effect with less material used (Das et al., 2012).

iii. NON NICOTINIC AGENTS:

a. Buproprion: It is a drug that is primarily used as an atypical antidepressant and also for smoking cessation by reducing the side effects of withdrawal. Marketed as Wellbutrin, Budeprion, Prexaton, Elontril, Aplenzin. Buproprion selectively inhibits neuronal reuptake of both the neurotransmitter dopamine and noradrenaline. Studies have shown that low doses of buproprion in rats block the rewarding effect of nicotine as assessed by intracranial self stimulation threshold and reverse the negative affective actions of nicotine withdrawal (Benowitz, 2008). It also inhibits the nAChR but shown biorhythmic side effects such as nausea, headaches and weight loss (Sirota et al., 2013).

b. Varenicline: It acts on nicotinergic α4β2 acetylcholine receptor that attenuates the craving for smoking and inhibiting affirmative effect of further nicotine taken up from smoking cigarettes. Users have reported various side effects like dreams, sleep disturbances, headache, nausea, dizziness, fatigue and gastrointestinal complaints. Varenicline is proscribed for use by children, adolescents, pregnant women and by smokers with psychological problems due to some cases of suicidal behaviour and feelings. It interacts with cimetidine, warfarin, and nicotine replacement drugs (Batra, 2011). Studies showed that it stimulates α4β2 nAChRs to maintain a moderate level of dopamine release, which reduces craving and withdrawal symptoms during abstinence from smoking and also blocks the reinforcing effects of nicotine obtained from cigarette smoke in the case of relapse (Benowitz, 2008).
c. Clonidine: It is α2-adrenergic receptor agonist. It acts on brain to reduce sympathetic neural outflow that results in symptoms like sedation and anxiolysis, as well as potential hypotension, bradycardia, and dry mouth. It brings calming and anxiolytic effects to the subject who is trying to quit smoking (Benowitz, 2008). Its role in preparing opiate dependent patient for transition from opiate agonist to naltrexone therapy has been documented. Studies have shown that clonidine reduces the cost and duration and cost of withdrawal processes (Bond, 1986). Studies have also shown that the success rate in clonidine treated subjects which is verified by serum clonidine concentration is more than twice as compared to placebo treated subjects (Glassman et al., 1988).

d. Nortriptyline: It is an antidepressant which acts by inhibiting the reuptake of nor epinephrine (noradrenaline) and, to a lesser extent, serotonin with negligible effects on dopamine reuptake. It has antagonistic effects at a variety of receptors: Strong: H₁, Moderate: 5-HT₂, α₁-adrenergic, mACh, Weak: 5-HT₁ (Kennedy et al., 1997). Used in combination with transdermal nicotine as it increases its effect (Kumar et al., 2011). Studies show that it improves the somatic signs of nicotine withdrawal in rodents (Wing and Shoaib, 2007).

e. Rimonabant:

It acts by selectively blocking the cannabinoid-1 receptors (Singh et al., 2006). It decreased nicotine self-administration and other behavioral effects (Xi et al., 2009; Dhippayom et al., 2011).

f. Nicotine Vaccines: It is a vaccine against nicotine induces antibodies against the nicotine molecule that prevents the drug from reaching neural receptors that produce the smoking like effects (Kumar et al 2011). Administration of nicotine vaccine, leads to gradual rise of antibody levels, which may attenuates nicotine withdrawal symptoms, and the possible persistence of the antibodies potentially provides long-term protection, preventing relapse (Fahim et al 2013).

g. Mecamylamine: It is used for lowering blood pressure. It is a non selective nicotine antagonist that has a prominent role in smoking cessation and attenuates
the pleasing affects of nicotine. At higher doses, it produces drowsiness, hypotension and constipation (Dasgupta et al., 2012). It is also used to precipitate nicotine withdrawal symptom in animal studies for evaluating the efficacy of new compounds for treatment of nicotine dependence (Singh et al., 2013).

2.5.2 NON PHARMACOLOGICAL MEASURES:

a. Novel therapies:

One of the novel approaches for nicotine dependence is immunization against nicotine. Antibodies produced by vaccine attach with nicotine circulating in the blood, thus prevent it from reaching the nAChRs in the brain and abolish nicotine addiction. It is well tolerated and safe method, in spite of not a success appreciably due to increase uninterrupted abstinence rates (Kumar et al., 2011).

b. Anti-smoking vaccine (Nic VAX):

NicVAX32, is a new anti-smoking vaccine currently used in clinical trials and approved by US FDA. NicVAX32 is a nicotine coupled vaccine anticipated to reduce substantial dependence to nicotine addiction as per National Institute on Drug Abuse. It made up of the hapten 3'-aminomethylnicotine entity, which has been conjugated with *Pseudomonas aeruginosa* exoprotein A (Kumar et al., 2011).

c. Green smoke electronic cigarette:

It is better than conventional cigarettes and is officially permitted. Green smoke electronic cigarettes produce the same palpable feeling, oral fascination that smokers yearning and satisfying their tobacco cravings as well. In e-cigarette, no tobacco product is used or burn, but one can inhale from it, as it activates a “flow censor” which produces water vapour containing propylene glycol, nicotine and a fragrance of tobacco. In these no cancer producing chemical agents are used and it only fixes nicotine for producing desired effect (Kumar et al., 2011). In nicotine-free cigarette, black and red pepper capsaicinoids are used in place of
nicotine. Nicotine-free cigarette and nicotine patches could offer the vital anti-smoking strategy (Kumar et al., 2011).

d. Behavioral Treatments:

Behavioral interventions can play a fundamental role in nicotine addiction treatment. Behavioral methods are employed to (a) Set up challenging coping responses (b) Build up self-monitoring of smoking behavior, (c) Produce an hatred to smoking (d) search out high risk reversion conditions. Factors like family and friend support, use of coping skills, avoiding smoking environments and company of smokers helps in prevention of relapse of smoking and tobacco use in nicotine addicts. Addicts must develop cognitive and behavioral methods for prevention of relapse in crisis or critical conditions (Kumar et al., 2011).

e. Diet:

During and after cessation of smoking one can courage nicotine addicts to follow a regular exercise regimen as preventive measure to overcome avert effect of addiction and must take low calorie diet so that they can check their weight gain (Kumar et al., 2011).

f. Activity:

Physical workout and exercise helps a lot in smoking cessation, as it helps to control weight gain and also lessen nicotine withdrawal symptoms in addicts (Kumar et al., 2011).

g. Hypnosis:

Hypnotism is said to be an excellent aid in helping a user to finally break the habit of smoking (Kumar et al., 2011).

h. Acupuncture:

It is recent method used for the treatment of smoking cessation with no side effects. In these days, acupuncture based on laser therapy i.e without use of
needles has been developed as preventive measures in nicotine addicts (Kumar et al., 2011).

**i. Motivational Therapies:**

Motivating smokers by telling them the benefits of quitting smoking both on health and financial ground, like saving a lot of money after quitting etc. Books, websites and motivational lectures can be alternative way to motivate addicts to quit smoking (Kumar et al., 2011).

**j. Quit meters:**

Computer aided programs keeps a record of quits statistics in the form of “quit-time”, cigarettes not smoked, and money saved (Kumar et al., 2011).

**k. Herbal treatments:**

**Mucus-Clear:** Alternative medication like Homeopathic medication clears phlegm a chunky viscous fluid secreted by the mucous membranes of the respiratory tract and relieves throat congestion for smooth flow of air.

**Crave-Rx Drops:** These drops supports in elevation of mood and feeling of well-being while quitting smoking.

**NicoTonic:** Homeopathic medications relieve the effects of worry, anxiety, stress and tension and also improve nervous system health.

**Rx-Hale:** It provides supports to the central nervous system while quitting smoking (Kumar et al., 2011).

**2.6 PROBLEMS WITH CURRENT CLINICAL STRATEGIES:**

The current drugs used in the clinic to treat nicotine dependence have been shown to just contain the symptomatology of the withdrawal syndrome rather than affecting the pathophysiological course of the disease and various adverse effects linked with them (Dasgupta et al., 2012). Therefore, research is being carried out
in order to find out pharmacological approaches that might ameliorate the problem of nicotine dependence (Berrettini and Lerman, 2005).

**2.7 ROLE OF NEURONAL nAChRs IN MOLECULAR MECHANISM OF NICOTINE DEPENDENCE:**

Neuronal nicotinic acetylcholine receptors are ligand-gated cation channels that are activated exogenously by the $3^0$ (tertiary) alkaloid i.e nicotine and the endogenously by agonist of nAChRs i.e acetylcholine (ACh), a prominent neurotransmitter of central nervous system (Albuquerque et al., 2009). nAChRs are part of super family of Cys-loop ligand-gated ion channels that include receptors for glycine, GABA [$\gamma$-amino butyric acid (the GABA$_A$, and GABA$_C$ receptor)] (Bernhard et al., 2004) and 5-hydroxytryptamine (5-HT$_3$) (LeNovere and Changeux, 1995; Changeux and Edelstein, 1998). These ionic channels have like structure and function and all the subunit of this family have a pair of disulfide bonded cysteine amino acid alienated by thirteen residues (Cys-loop) in their extracellular amino terminus (Karlin, 2002). nAChRs mediate fast and direct synaptic transmission at neuromuscular junction and autonomic ganglia. nAChRs are expressed in the soma in neurons where they modulate excitability directly. nAChRs are also located in presynaptic terminal where they facilitate calcium dependent release of neurotransmitters like adrenaline, glutamates, dopamine, serotonin and many others. This process takes place by indirect or direct mechanism. In indirect mechanism of nicotine dependence, sodium influx causing membrane depolarization and activates voltage gated calcium channel or by direct mechanism where activation of voltage gated calcium channel or by direct influx of calcium channel synaptic and non neuronal transmission of nAChRs leads to nicotine dependence (Hendrickson et al., 2010). Studies show that repeated exposure of nicotine to nAChRs produce dependence by sensitization (Mihov and Hurlemann, 2012). Nicotine dependence is induced by two ways: drug could have increased pharmacological effect for example when it increases the number of nicotine in nicotinic acetylcholine receptors or strengthening their coupling to effecter proteins (Berke and Hyman, 2000).
2.7.1 The mechanism of nicotine dependence described above can be summarized as:

Binding of nicotine to the nicotinic cholinergic receptor opens voltage-gated calcium channels by inducing a change in the conformation of α and β subunits which result in the altered brain concentrations of dopamine, serotonin, noradrenaline, γ aminobutyric acid, glutamate, acetylcholine, and endorphins. Nicotinic cholinergic receptors are widespread in the central and peripheral nervous systems; however, nicotine dependence is particularly affected by receptors localized in the ventral tegmental area, which promote release of dopamine in the nucleus accumbens and prefrontal cortex. The initial pleasurable sensations produced by nicotine are positively reinforcing. Prolonged exposure to nicotine leads to neuroadaptation, a process by which the number of binding sites on the nicotinic cholinergic receptor change contributing to physical dependence (Danovitch, 2011).

2.8 NEUROCHEMISTRY OF NICOTINIC RECEPTOR AND THEIR SUBUNITS:

Nicotine influences neuronal activity, synaptic communication, and ultimately behavior, through its effects on nicotinic receptors (Hendrickson et al., 2013). These receptors have two or more agonist binding sites which results in conformational change that leads to ion flux through the pore, inducing a depolarization and increased excitability (Keath et al., 2007). Nicotine ability to activate nicotine acetylcholine receptor depends on subunits that make up receptors. The nAChRs are ligand-gated ion channels, consisting of 5 membrane straddling subunits that are allosterically regulated (Picciotto and Kenny, 2013). nAChRs exist in 12 isoforms (Variant forms) labeled α2 to α10 and β2 to β4. Each nicotinic acetylcholine receptor made up of five subunit molecules set in a ring around the central channel that opens to release ions when receptor gets activated (D’Souza and Markou, 2011). The principal nicotinic acetylcholine receptor subtypes in human brain are those containing α4 and β2 subunits (denoted as α4β2 nAChRs) (Picciotto and Kenny, 2013). In homomeric nAChR all subunits are the same for example all contain α7. Heteromeric nAChRs have
mix subunits, for example two $\alpha_4$ and three $\beta_2$ subunits. Mix subunits give receptors its distinct pharmacological properties, including response to nicotine stimulus (D’Souza and Markou, 2011). nAChRs oscillate between four dominant states: a). The resting state (R: channel closed and agonist binding site unoccupied), b). The active state (A: channel open), c). The desensitised state (D: channel closed and agonist bound with high affinity) and d). The inactive state (I: a long-lasting desensitized state) (Benowitz, 2008).

2.8.1 Resting of nAChRs:

nAChR are in resting state and non-functional till they are attached to agonist i.e nicotine (Govind et al., 2009). Opening of nAChRs binding an agonist like nicotine transiently alter the conformation of the channel. The open channel permeates mainly sodium and potassium ions, but calcium also carries about 1%–10% of the current, depending on the nAChR subtype (Dani and De Biasi, 2001). Closing and Desensitization of nAChRs after opening for milliseconds, channel closes to resting state or it can also enter desensitization state due to some circumstances in which channel become closed and unresponsive to agonist (Dani and De Biasi, 2001; Govind et al., 2009).

Nicotine acetylcholine receptors are integral membrane protein and prototypic members of ligand gated ion channels super family which have precursors in prokaryotic world. They are formed by assembling of 5 transmembrane subunits which are obtained selectively by pool of 17 homologous polypeptide ($\alpha_{1-10}$, $\beta_{1-4}$, gamma, delta, and epsilon) in central nervous system. In the peripheral nervous system, nicotine acetylcholine receptors helps to mediates synaptic transmission of various impulses at the ganglia and neuromuscular junction. nAChRs are also present in keratinocytes, epithelia, macrophages, non-neuronal and in nonmuscle cells (Kalamida et al., 2007). nAChRs family is divided into two groups:

2.8.2. Acetylcholine muscle receptors:
They are present at the neuronal nicotinic acetylcholine receptors, which are distributed throughout both the central nervous systems and peripheral nervous system and also located at the skeletal neuromuscular junction where they mediate release of various neurotransmitters for conduction of nerve impulses and their transmission.

2.8.3. Muscle-type nAChR:

These receptors are pentameric complexes with subunits like; α (alpha), β (beta), δ (delta) and γ (gamma) in fetal receptors in the adult forms α, β, γ and € with two α1 subunits in each pentamer (Rahman et al., 2008).

nAChRs are located both presynaptically and postsynaptically in CNS. Presynaptically, nAChRs helps to regulate the release of various neurotransmitters. Postsynaptically, the nAChRs helps for swift synaptic transmission of impulses. Agonist like nicotine binding also involves a cation interface with the nAChR. It has strong interaction with neuronal nicotinic acetylcholine receptor than Muscle-type nicotinic acetylcholine receptors due to difference in an amino acid near Tryptophan synthase beta chain. At pH more than 5, nicotine got deprotonated, which enhances its ability to cross blood brain barrier (Hegde et al., 2012).

The acetylcholine binding sites of the nAChR made up of complementary and principal components. In case of homo pentameric (i.e. α7) receptors the same subunit carries on its opposite sides, at the interface of these subunits complete ligand-binding. In case of heteropentameric receptors α (α2, α3, α4 or α6) subunits carry the principal components, and β subunits (β2 or β4) carry the complementary components of ligand-binding sites (consequently hetero pentameric receptors have 2 similar acetylcholine binding sites per receptor molecule) (Domea et al., 2010). Major source of interventions in the mid brain occur. Dopamine area arises from nearby pedunculo pontine tegmentum (PPT) and laterodorsal tegmentum (LDT) which are loose collections of cholinergic neurons scatter along with glutamatergic and GABAergic neurons. The main projection of PPT is toward substantia nigra compacta and LDT project to VTA.
LDT and PPT both are responsible to the event of drug taking behavior. Studies have shown that lesions in PPT reduces nicotine self administration. VTA gets strong excitatory glutamate from prefrontal cortex, that excitation is mainly onto dopaminergic neurons that project back to the cortex and not the nucleus accumbens. It is found that PPT and LDT provide direct glutamatergic projections to dopamine neurons projecting nucleus Accumbens (Dani & Harris., 2005).

Figure 1: Various nicotine receptors subunits involved in somatic and affective withdrawal symptoms and their respective location in Brain:

A. β2 nAChRs: This subunit plays a prominent role for the depolarization dopamine cell bodies in the VTA by nicotine to increase their firing rate (Picciotto and Kenny, 2013). The behavioral effect of nicotine which is its self administration is mediated by this nicotine receptor subunit (Benowitz, 2008). Social interaction, considerations, decision making and rewarding effect of nicotine dependence are due to β2 nAChRs (Changeux, 2010).

B. β2 subunit: β subunit gene eliminates the behavioral effects of nicotine; reinserting the gene into the ventral tegmental area restores behavioral responses to nicotine (Benowitz, 2010).

C. α7 nAChRs: Glutamate input to DA neurons can be accelerated by nicotine neurons through α7 nAChRs (Picciotto and Kenny, 2013). It is similar to
homo-oligomeric $\alpha_7$ receptors which is studied in exogenous expression systems, but it rarely form agonist destabilizes heterooligomeric nAChRs. It also has rapid activation and desensitization kinetics (Pidoplichko et al., 1997). These nAChR subunits are responsible for schizophrenia, due to activation of nAChRs, through glutamamnergic excitation (Marks et al., 1986). The $\alpha_7$ subtype is mediate cardiovascular effect of nicotine; it is thought to be involved in rapid synaptic transmission and may play a important role in learning and sensory gating (Benowitz, 2008). They have highest calcium permeability. $\alpha_7$ subunit shown voltage-sensitive inhibition of the ion channel by intracellular polyamines and Mg$^{2+}$, so that they do not conduct current when the membrane is in depolarized state (Rahman et al., 2008). $\alpha_7$ subunits of nAChRs have allosteric-binding sites for their positive modulators (Dome et al., 2010).

D. $\alpha_7$ and $\alpha_4\beta_2$ subunits: These subunits of nAChRs have allosteric-binding locations for their modulators both positive and negative in nature (Domea et al., 2010). These subunits are responsible for modulation of glutamate release (Wang and Sun, 2005).

E. $\alpha_4/\beta_2$ nAChRs: Activation of these subunits leads to depolarization of DA neurons in the VTA (Garduño et al., 2012; Picciotto and Kenny, 2013). $\alpha_4\beta_2$-subunits of nAChRs shown depression-like state (a negative affective), during which they showed no somatic signs of nicotine withdrawal symptoms but shows less responsive to electrical stimulation of their reward system (D’Souza and Markou, 2011). Pleasurable effects of nicotine are due to these beta subunit receptors (Jain et al., 2008). Craving of nicotine symptom occur by desensitization of these receptor (Benowitz, 2008). These receptors are expressed in human embryonic kidney cell, and show a dose-dependent loss of Ca$^{2+}$ entry when exposed to 0.1–10 mM nicotine for an hour or longer (Govind et al., 2009).

F. $\alpha_4: \beta_2$ subunit: These subunits in the ratio of 2:3, shown up-regulation after prolong exposure of nicotine. Upregulation of $\alpha_4\beta_2$ subunit of nAChRs in nicotine dependent, critically influence the nicotine-induced desensitization (Rahman et al 2008). It is present in midbrain dopaminergic neurons. $\alpha_4\beta_2$ receptors, formed a high affinity binding complexes that are pentameric,
trafficked to the cell surface, and produced acetylcholine evoked currents (Walsh et al., 2008). Heteromeric α4β2 receptors subunits of nAChR is pentameric membrane protein consists of α (two) and β2 subunits (three), found throughout the brain with two agonist binding sites. These subunits are present in mesolimbic dopamine neurons that control the release of dopamine and other neurotransmitters. α4β2 receptors subunits of nAChR have great affinity for binding with nicotine. α7 and α4β2 subunits of nAChR have allosteric-binding sites for their modulators both positive and negative in nature (Domea et al., 2010).

G. α6 nAChRs: These subunits are responsible for nicotine self-administration (Picciotto and Kenny, 2013). It is found in less than 25% of GABA neurons (Mansvelder et al., 2008). Studies show that these subunits contribute to spontaneous locomotor behavior (Changeux, 2010). Studies show role of these subunits in ascending dopaminergic pathways and also in more elaborate, top-down ‘gating’ strategies involving attentional control (Changeux, 2010). Studies show that these subunits of nAChRs are responsible for rewarding effect of nicotine dependence (Changeux, 2010). Previous study showed that α6 subunits have been difficult because α6 subunits appear to aggregate instead of forming functional pentameric receptors (Walsh et al., 2008).

H. β4 nAChRs:

It plays a prominent role in appetite-suppressing effects of nicotine. β4 subunit of nAChRs is located in POMC (proopiomelanocortin) neurons in the arcuate nucleus of the hypothalamus (Picciotto and Kenny, 2013). β4 nAChRs subunit is responsible for withdrawal symptoms of nicotine dependence (Dani et al., 2011)

I. α5/β2 nAChRs: α5/β2 subunit of nAChRs presents on cortical glutamatergic projection neurons to the thalamus region of the brain. These subunits are important in maturation of glutamatergic circuit in thalamus. It plays a prominent role in normal adult task in passive avoidance test, along with this in a somatosensory aversive learning task in rats (Picciotto and Kenny, 2013).
J. α5, α5β4, α4β2 nAChRs: Stimulation of α5 and α5β4 nAChRs is important for the anxiogenic like behavioral effects of nicotine (Picciotto and Kenny, 2013). α5 is found in less than 25% of GABA neurons (Mansvelder and McGehee 2000). α5 increases sensitivity to nicotine activation of α3β2 but not α3β4 nAChRs (Kuryatov et al., 2013). β3 and α5 subunits of nAChRs are exclusive in this categorization because they hold neither the principal nor the complementary component of the ACh-binding site; consequently, they are considered accessory subunits. Study showed that absence of up regulation of α4β2α5 receptor subunit after chronic nicotine administration in the rodent brain in contrast to the massive up regulation of simple α4β2 receptor subunit (Domea et al., 2010). This subunit is responsible for withdrawal symptoms of nicotine dependence (Dani et al., 2011).

K. α2 nAChRs: α2 nAChRs subunits are not found in GABAergic neurons (Mansvelder and McGehee, 2002).

L. α3 nAChRs: It is present in midbrain dopaminergic neurons (Mansvelder and McGehee, 2000; Walsh et al., 2008).

M. α3β4 nAChRs: It mediates the cardiovascular effects of nicotine (Benowitz, 2008).

N. α5 nAChRs: Studies showed that these subunits of nAChRs are responsible for rewarding effect of nicotine dependence (Barik and Wonnacott, 2009; Changeux, 2010). α5 subunit combined with α4β2, increases calcium conductance seven times. Activation of α5 gene variants also alter nicotine responsiveness in cultured human cells (Benowitz, 2010).

O. β3 nAChR: These subunits, in which the tyrosine residues of the complementary binding site loop E are replaced by phenylalanine, do not participate in agonist binding (Barik and Wonnacott, 2009). β3 nACh receptor is unique in this classification because they carry neither the principal nor the complementary component of the ACh-binding site. Consequently, they are considered accessory subunits (Domea et al., 2010). β3 subunits located in
striatum alter motor activity by means of modulating DA release (Wang and Sun, 2005).

**P. α4 nAChR:** nAChR on VTA is mainly composed of α4 sub unit (Dani & Harris, 2005). Studies by Changeux, (2010) showed that these subunits contribute to spontaneous locomotor behavior and rewarding effect in nicotine dependent rodents. These subunits are present in ascending dopaminergic pathways and also work in more elaborate, top-down ‘gating’ strategies involving attentional control (Changeux, 2010). This subunit is required for the transition from tonic to phasic firing that is crucial for reinforcement (in particular, in response to reward-predicting stimuli and unpredicted rewards) (Changeux, 2010).

**Q. α6β2 nAChR:** It is formed high affinity epibatidine binding complexes that are pentameric, trafficked to the cell surface, and produced acetylcholine evoked currents. α6β2 receptor up-regulation required, higher nicotine concentrations than for α4β2 but lower than for α3β2 receptors. α6β2 receptor up-regulation occurred 10-fold faster than for α4β2 and slightly faster than for α3β2 during chronic nicotine exposure (Walsh et al., 2008).

**R. α3β2:** This subunit is formed by high affinity epibatidine binding complexes that are pentameric, trafficked to the cell surface and produced acetylcholine evoked currents during acute nicotine exposure (Walsh et al., 2008).

**S. β2 subunits:** During acute nicotine exposure, β2 subunit controls the GABA release and responses to ACh by Dopamine neurons in mesencephalon (Wang and Sun, 2005).

### Table 1: nAChR influences on neurotransmitter release.

<table>
<thead>
<tr>
<th>Neurotransmitter Released</th>
<th>Brain region</th>
<th>nAChRs involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>VTA</td>
<td>α4β2*</td>
</tr>
<tr>
<td></td>
<td>NAcc</td>
<td>α4*, α6*, α6β2*</td>
</tr>
<tr>
<td></td>
<td>PFC</td>
<td>α4β2*, α7</td>
</tr>
<tr>
<td>Glutamate</td>
<td>VTA</td>
<td>α7</td>
</tr>
<tr>
<td></td>
<td>NAcc</td>
<td>β2</td>
</tr>
<tr>
<td></td>
<td>PFC</td>
<td>α4β2*, α7</td>
</tr>
</tbody>
</table>
Various neurotransmitters released in response to nicotine administration, the brain areas where this release is known to occur, and nAChR subtypes known to facilitate this action. VTA; ventral tegmental area; NAcc; nucleus accumbens; PFC; prefrontal cortex; IPN; interpeduncular nucleus; GABA; γ-aminobutyric acid; MHb; medial habenula.

*The asterisk denotes the potential presence of other nAChR subunits.

### 2.9 Nicotine induced neuroadaptations

Nicotine addiction involves long-lasting malfunctioned adaptive changes including development of disruptive nicotine stimuli associations. Nicotine-induced neuroplasticity underlies the development of tobacco addiction, in regions such as the hippocampus, the ability nicotine to enhance cognitive capabilities. Nicotine dependence is accompanied by neuroadaptive changes that occur in the circuits underlying emotion and motivation (Koob and Volkow, 2010). Preclinical studies of nicotine dependence suggests a important neurotransmitter interactions between various transmitter like glutamate, gamma-aminobutyric acid (GABA), cholinergic and dopamine in the ventral tegmental area, central nucleus of the amygdala and the prefrontal cortex (Markou, 2008). Neuronal nAChR up regulation is a significant adaptive change due to continous exposure to nicotine and leads to addictive properties of nicotine (Wonnacott, 1990). Nicotine desensitizes nAChRs and renders them insensitive and leads to increase in upregulation of receptors for maintaining circuit-level homeostasis (Fenster et al.,

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Brain Area</th>
<th>Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>VTA</td>
<td>α6β2*</td>
</tr>
<tr>
<td></td>
<td>NAcc</td>
<td>α4β2*</td>
</tr>
<tr>
<td></td>
<td>Amygdala</td>
<td>α3β4*, α7</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>MHb</td>
<td>α3β4*</td>
</tr>
<tr>
<td></td>
<td>IPN</td>
<td>α3β4*, α3β3β4</td>
</tr>
<tr>
<td>Nor epinephrine</td>
<td>Hippocampus</td>
<td>α3β4*, β4* (Rat), α6β2β3 (Mouse)</td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>α3β2, α6*</td>
</tr>
</tbody>
</table>
This upregulation of nAChRs differs among receptor subtypes, due to chronic exposure for nicotine leads to changes in receptor assembly, trafficking, and degradation, varies among brain regions for the same subtype (Gentry and Lukas, 2002; Lester et al., 2009). Isomerization of surface nAChRs to high-affinity nicotinic sites is due to binding with nicotine as agonist for active sites (Buisson and Bertrand, 2002; Vallejo et al., 2005). Repeated nicotine exposure produces heterologous neuroadaptation, which alters the cholinergic inputs, modulates various neurotransmitter releases and cholinergic function. Nicotine increases AMPA/NMDA current ratios at dopamine neurons (Saal et al., 2003; Placzek et al., 2009; Gao et al., 2010) and other locations involved in drug-associated memory (Dani and Biasi, 2001; Kauer and Malenka, 2007). In the nucleus accumbens (NAcc), administration of nicotine leads to an increase in high affinity Dopamine (DA) D2 receptors (Novak et al., 2010), which leads to an increase in G-protein coupled DA D2 receptors and resulting in DA super sensitivity in nicotine treated animals (Briand et al., 2008). Nicotine self-administration also decreases expression of the cystine–glutamate exchanger, in the NAcc and ventral tegmentum area (VTA), and decreases the glial glutamate transporter, in the NAcc (Knackstedt et al., 2009). Nicotine also inhibits proteasomal function by altering the scaffolding proteins at Synaptic junction (Hwang and Li, 2006; Rezvani et al., 2009). Nicotine exposure also alters the functioning of endogenous opioid system that regulates both negative and positive motivational and affective states (Berrendero et al., 2005; Trigo et al., 2010; Hadjicostantinou and Neff, 2011). Opioid peptides like dynorphins and enkephalins affect DA function in the VTA and the striatum (Di Chiara and Imperato, 1998). Dopamine controls the formation of striatal dynorphin (Devine et al., 1993; Di Chiara and Imperato, 1998) and enkephalin (Spanagellet et al., 1991, 1991; Penteny and Gratton, 1991) by affecting their transcription (Angulo and McEwen, 1994). Nicotine affects these system and cellular level interactions by altering synthesis and release of opioid peptides in a time and peptide-specific manner that participate in the nicotine-withdrawal
syndrome (Steiner and Gerfen, 1998). Agonist (nicotine) based nAChR activation affects the release of various neurotransmitter (Kenny, 2011; Picciotto, 1998). Chronic nicotine exposure causes major alterations in brain neurotransmission system which is responsible for system and between systems adaptations.

![Diagram]

Figure 2: Tubular flow of events showing nicotine induced neuroadaptations:
2.10 NEURAL BASIS OF NICOTINE WITHDRAWAL:

![Brain Diagram]

Figure 3: Various parts of brain involved in Nicotine withdrawal syndrome:

Sudden stoppage of nicotine alters the neurochemistry of nAchRs in addicted brain that triggers the affective and somatic signs of withdrawal. Acute withdrawal syndrome induced by nicotine, decreases activity of the mesolimbic DAergic system by reducing dopamine release and an increase in dopamine reuptake (Weiss et al., 1992; Hildebrand et al., 1998; Carboni et al., 2000; Rada et al., 2001; Rahman et al., 2004; Duchemin et al., 2009; Hadjiconstantinou et al., 2011). Nicotine induced withdrawal symptoms appeared due to deficits in dopamine transmission in NAcc and increase upregulation of dopamine transporter in the prefrontal cortex (PFC) (Thierry et al., 1976; Bradberry et al., 1991; Inglis and Moghaddam, 1999; Kawasaki et al., 2001; Broersen et al., 2000; Carboni et al., 2000). This increase in dopamine level in PFC trigger by stressful and aversive stimuli that contributes anxiety like behaviors in nicotine dependent subjects, to attenuate aversive stimuli and its effect smoking and administration of drug of abuse plays a calming effect and role of potent negative reinforcer in addicts which leads to increased craving and relapse (Pomerleau and Pomerleau, 1984; Cohen and Lichtenstein, 1990; Perkins and Grobe, 1992; Carey et al., 1993; Doherty et al., 1995; Parrott, 1995; Jorenby et al., 1996; Brown et al., 2001; Sinha, 2001; Morissette et al., 2007). Extended amygdala and the hypothalamic-pituitary-adrenal axis plays an important role in negative affective states linked
with nicotine induced withdrawal syndrome (Koob, 2010). Increased levels of Stress hormone corticosterone and corticotropin-releasing factor (CRF) observed during nicotine induced withdrawal symptoms. Study by George et al (2007) shown that level of CRF increased by 500 times in central nucleus of the amygdala (CeA), after nicotine withdrawal is precipitated with the non selective nAChR antagonist mecamylamine. Intracerebral injection of a CRF1 receptor antagonist into the CeA precipitates anxiety-like behavior in nicotine dependent rodents (George et al., 2007). Nicotine induced neuroadaptations occur at various brain sites like amygdala (CeA), nucleus of the striatalis, VTA, NAcc and IPN and affect release of several neurotransmitter and neuropeptide systems that disrupts nicotine withdrawal syndrome (George et al., 2007; Koob, 2010). The opioid peptides, serotoninergic and noradrenergic systems are known to modulate various mechanisms of nicotine withdrawal (Slotkin and Seidler, 2007; Fletcher et al., 2008; Bruijnzeel et al., 2010; Semenova and Markou, 2010; Hadjiconstantinou et al., 2011).

![Figure 4: Effect of Nicotine on various modulators of behaviour. Adapted from (Picciotto, 1998) under License Number: 3551140470929 with permission from publisher.](image-url)
2.11. NEUROTRANSMITTERS AND MEDIATOR SYSTEMS INVOLVED IN NICOTINE WITHDRAWAL:

Acute and continuous exposure of nicotine or drug of abuse increases the release of neurotransmitter dopamine from dopaminergic (DAergic) neurons that originating from ventral tegmental area (VTA), nucleus accumbens (NAcc), prefrontal cortex and hippocampus, is the most important cause for nicotine addiction (Balfour, 2008). Binding of Nicotine with nAch receptors leads to activation of the VTA that result in DA release in the NAcc. Continuous nicotine exposure results in upregulation of nAChRs at different rates that become desensitized and provoke glutamate-mediated excitation that increases the firing frequency of DAergic neurons to release dopamine and diminishes GABA-mediated inhibitory tone for enhancing the responsiveness to nicotine (Benowitz, 2010)

Figure 5: Schematic depiction of nicotine–acetylcholine–glutamate–GABA–dopamine interactions involved in mediating effects of nicotine withdrawal. Adapted from Mansvelder and McGehee (2002).

1. Dopamine:

Dopamine is an excitatory neurotransmitter which is classified into two type of receptors D1 and D2 based on their pharmacological action, that are further subdivided into their subtype: D1 (D1 and D5) and D2 (D2, D3, D4) mostly present in central nervous system. Both D1 and D2 receptors act opposite to each other with respect to their activation. Activation of D1 family receptors
increases cyclic adenosine monophosphate 3, 5 monophosphates (cAMP) by enzyme adenylate cyclase (AC) through stimulatory Gs protein of g protein couple receptors. Whereas, the activation of D2 receptors decreases cAMP through a G inhibitory protein pathway. Intercellular cAMP leads to the activation of enzyme protein kinase and G-protein receptor kinase-3 (GRK3) that phosphorylates various receptors and channels of dopamine which leads to the activation of transcription factors like cyclic adenosine monophosphate response-element binding protein (CREB) (Brunzell et al., 2003). Nicotine’s addictive properties are due to modulation of these intracellular transduction pathways and alteration in expression of gene product upon continuation activation of these intracellular pathways (Reddy et al., 2013).

2. Dopamine and Glutamate:

Excitatory role of N-methyl-D-aspartate (NMDA) receptors in the VTA on nicotine-induced increases release of dopamine in nucleus accumbens (Girault and Greengard, 2004). Acute administration of nicotine activates nAChRs located pre-synaptically on glutamatergic terminals, leading to increased evoked glutamate release which further increases the burst firing of these neurons and subsequent dopamine release in the nucleus accumbens (Watkins et al., 2000). Studies have shown that nicotine elevates extracellular glutamate levels in a number of brain regions including the cerebral cortex, dorsal striatum, hippocampus, hypothalamus, locus coeruleus and cerebellum which result, in excitatory postsynaptic currents and neural activity increase via mGluR-mediated mechanisms including the induction of hippocampal LTP that leads to long-lasting synaptic plasticity in numerous brain regions (Bonsi et al., 2005), which may promote addiction to this substance and adaptive changes in the expression of various proteins related to glutamate neurotransmission occur. Chronic nicotine self-administration in rats also increases GluR2/3 expression in the VTA but does not induce changes in NR2A, NR2B, or GluR2/3 levels in the NAcc but an up regulation of the glutamate transporter EAAT2 has been reported, the expression of mGluRs and Homer1 and Homer2 mRNA in the amygdala, NAcc and VTA are altered by nicotine although many of these changes are only transient. The
adaptive changes in glutamate transmission produced by nicotine may be age-related, which may provide a neural basis for the enhanced vulnerability to nicotine addiction during adolescence in both humans and animals (Dunayevich et al., 2008). Thus, nicotine may produce age-dependent adaptations in glutamatergic transmission (Gass and olive, 2008). Dopamine and glutamate are widely distributed in areas of brain like cortex, limbic system and basal ganglia that are responsible for physical behavior of nicotine dependence like motivation, learning and memory. Coordinated behavior of dopamine and glutamate as seen in nicotine dependence is due to its mechanism through their DA D1, glutamate, N-methyl- D-aspartate (NMDA) and α-amino-3-hydroxy-5- methylisoxazole-4-propionic acid (AMPA) receptors which are further responsible for the intracellular pathways and leads to nicotine dependence leading to adaptive changes in gene expression and results in synaptic plasticity (Fehr et al., 2008; Reddy et al., 2013).

3. Gamma aminobutyric acid (GABA) and Dopamine Interaction:

Inhibitory GABAergic afferents are intricately linked with the dopaminergic neurons in ventral tegmental area and mediates its physiological functions. Medium spiny GABAergic neurons in the nucleus accumbens channelize the GABAergic inhibitory afferent impulses in VTA to cause the inhibition of mesolimbic dopamine release (Watkins et al., 2000).

Neurons present in various brain areas such as nucleus accumbens, pedunculopontine tegmental nucleus, ventral pallidum and neurons located within VTA release Gamma aminobutyric acid in the VTA that effects the reinforcement and reward effect by decreasing the quantity of dopamine in mesolimbic dopaminergic neurons. Endogenous GABA acts via ionotropic GABA_A and GABA_C receptors and metabotropic GABA_B receptors. In nicotine-naive animals, nicotine administration increases level of gamma aminobutyric acid by activating excitatory subunits of nAChRs i.e α4β2, present on ventral tegmental area of GABAergic neurons. Thus initially, nicotine-induced GABA release limits the rewarding effects of nicotine but lead to the desensitization of α4β2-containing nAChRs on GABAergic receptors. This desensitization decreases nicotine
mediated Gamma aminobutyric acid release and leading to decreased inhibition of dopaminergic neurons in the VTA and increased overall dopamine release in the NAc that assists in the reinforcing effects of nicotine. Compounds that increase GABAergic neurotransmission decrease both the reinforcing effects and reinstatement of cue-induced nicotine-seeking behavior in rodents. Thus, administration of various compounds or treatments that increases the Gamma aminobutyric acid transmission may inhibit relapse to tobacco smoking (D'Souza and Markou, 2011).

**Nigrostriatal Dopamine System:** When chronic nicotine administration takes place, it activates nigrostriatal dopaminergic system which is responsible for movement disorders related to nicotine dependence (Ziedonis and George, 1997).

**Mesolimbic Structures (Nucleus Accumbens, Ventral Tegmental Area):** On chronic exposure of nicotine these brain parts get activated which brings physiological alteration in emotional expression, including positive symptoms of psychosis (delusions, hallucinations and thought disorder) and in drug reinforcement and reward (Ziedonis and George, 1997).

**Mesocortical system:** Larger and repeated exposure of nicotine activates these ventral tegmental projections to the prefrontal cortex. It gets hypo functional in chronic schizophrenia caused by chronic exposure of nicotine (Ziedonis and George, 1997).

**4. Serotonin (5-Hydroxytryptamine) and Nicotine Addiction:**

Projection of serotonergic neuronal track in dorsal raphe nuclei run to the dopaminergic area of midbrain, mesocorticolimbic system and hippocampus. 5-Hydroxytryptamine plays an important mediator in nicotine withdrawal. Serotonin receptors have been classified into three subclasses: 5-HT1, 5-HT2 and 5-HT3 receptors. The most abundant form of 5HT receptor in VTA is 5-HT1B (Reddy et al., 2013). Mannucci et al., (2007) proved that chronic administration of nicotine leads to increase in the density of 5 HT1A receptors in brain and reducing the effect of stress by acting on 5-HT neurons within the hippocampus (Mannucci et
al., 2007). Serotonergic system enhances the positive reinforcing effects of nicotine, but it has also been proved that it also act on negative reinforcing effects of nicotine withdrawal (Mannucci et al., 2007). Release of 5-HT is regulated by the auto receptors present in the nerve endings and hetero receptors located on dopaminergic, glutamatergic, GABAergic or cholinergic neurons that plays prominent role in nicotine addiction (Cloëz-Tayarani and Changeux 2006; Reddy et al., 2013).

5. Endogenous Opioid System and Nicotine Dependence:

The endogenous opioid system is a part of large and complex system of neurons in the nervous system. Important members of endogenous opioid system are the opioid peptide systems which produce active peptides from it which includes beta-endorphin, met and leu-enkephalins, dynorphins and recently discovered endogenous peptide nociceptin/orphanin FQ (N/OFQ). With any ligands opioid system activation produces their action by acting on its receptors which include mu, delta, kappa opioid and N/OFQ receptors. Various studies have shown that nicotine in tobacco smokers release enough brain opioid peptides by modulating mu opioid receptor. Mu opioid receptors are responsible for the decreased release of nicotine but with increased binding potential in amygdala, thalamus and VTA which are involved in the anticipation of reward of nicotine dependence. Further, findings show that nicotine enhances the level of endogeneous opioids peptides derived from preproenkephalin, which are found to be involved in antinociceptive effect induced by nicotine dependence (Xue and Domino, 2008).

6. Endocannabinoids and Nicotine Dependence:

The endocannabinoids system modulates the addictive properties of nicotine (Cippitelli et al., 2011). This system is responsible for the rewarding effects of cannabinoids, nicotine, alcohol and opioids, through the release of endocannabinoids in the ventral tegmental area of mesolimbic brain. Cannabinoid and nicotinic acetylcholine receptors are widely distributed in hippocampus and the amygdala part of the brain responsible for nicotine seeking behaviour.
Cannabinoid receptor activation has been shown to modulate nACRs, which promotes release of acetylcholine in various brain parts. Recent clinical trials have suggested that the CB1 cannabinoid antagonist rimonabant can be used for smoking cessation. Thus, CB1 cannabinoid antagonists could represent a new generation of compounds to treat nicotine addiction (Aydin et al., 2012; Maldonado et al., 2006). Chronic nicotine administration increased arachidonylethanolamide levels in the limbic forebrain and brainstem but decreased levels in the hippocampus, striatum, and cerebral cortex. CB1 receptor antagonist, rimonabant, decreased nicotine self-administration and conditioned place preference in rats, suggesting that endocannabinoid signaling is involved in nicotine reinforcement (Merritt et al., 2008).

7. Noradrenaline and Nicotine Dependence:

Nicotine dependence induced withdrawal syndrome is characterized by depression-like behaviour that may be mediated by dysregulation in norepinephrine transmission (Paterson et al., 2008). Nicotine mediated activation of nicotine acetylcholine receptor in post ganglionic sympathetic nerve endings enhances the level of noradrenaline in various tissues that indicates an exocytotic, calcium-dependent release of various neurotransmitter (Richardt et al., 1994). Noradrenergic neuron tract from brain stem nucleus tractus solitarius (NTS) to hypothalamic paraventricular nucleus (PVN) and amygdala (AMYG) are involved in nicotine related stress responses and craving that is mediated entirely through the effects of nicotine on glutamate afferents in NTS and NMDA receptors that, in part, stimulate NO production, resulting in activation of noradrenergic neurons (Zhao et al., 2007). Noradrenergic cell bodies in the nucleus accumbens and ventral tegmental area project afferents neurons to the A1 and A2 areas of the brain stem as well as locus coeruleus (LC). Noradrenaline acts through the various excitatory mechanisms, directly stimulate the dopamine cell firing and indirectly potentiate the activity noradrenergic neurons projecting to the prefrontal cortex (PFC) and affect the dopamine neurotransmission in the nucleus accumbens that plays a prominent role in nicotine dependence (Reddy et al., 2013).
8. Arginine-Vasopressin in Nicotine Dependence:

Cigarette smoking produces a rise in blood pressure associated with an increase in plasma catecholamines and other vasoactive hormones, including vasopressin in fairly large amounts (Marano et al., 1999; Caldwell and Young, 2006). Nicotine induces stress because of neuro-endocrine responses like release of arginine vasopressin (AVP) from parvocellular division of the paraventricular nucleus (PVN) and activation of the hypothalamo-pituitary adrenal (HPA) axis (Suzuki et al., 2009). Vasopressin receptor antagonist, SSR149415, suggest an innovative approach for the treatment of nicotine induced stress-related disorders (Griebel et al., 2002).

9. MAO–A and MAO-B in Nicotine Dependence:

Cigarette smoking causes a marked decrease in the levels of an important enzyme, monoamine oxidase (MAO) that is responsible for breaking down dopamine. The decrease in two forms of this enzyme MAO- A and B, results in an increase in dopamine levels. Although nicotine causes increase in brain dopamine, nicotine itself does not alter MAO levels. However, MAO in noted to affect dopamine release leading to nicotine dependence indirectly (Jain, 2003).

10. CREB (cyclic adenosine monophosphate response-element binding protein):

CREB is a cellular transcription factor which binds to DNA sequences called cAMP response elements (CRE), thereby increasing or decreasing the transcription of the downstream genes (Bourtchuladze et al., 1994). Chronic use of nicotine up regulates the µ opioid receptors and alters the transcription factor CREB which leads to nicotine dependence and the reinforcing behavior like craving and relapse (Dani and Harris, 2005). CREB is acting through calcium and Protein kinases A pathway in cortico-striatal region plays a major role in learning, this process is transduced by the glutamate and dopamine signals, respectively. Many genes which are responsible for the generation and phosphorylation of CREB are dependent on NMDA and dopamine D1 receptor (Grieder et al., 2012).
Drugs which are antagonize the NMDA and/or Dopamine D1 actions they inhibit the generation of CREB plays a good therapeutic agent in nicotine addiction (Reddy et al., 2013).

11. Neuropeptide Y:

Orexigenic neurotransmitter Neuropeptide Y (NPY) attenuates somatic withdrawal signs linked with nicotine dependence. Stimulation of NPY receptors prevents the reward function and somatic signs associated with nicotine withdrawal (Chen et al., 2005). NPY has been reported to decrease neuronal excitability by decreasing excitatory synaptic transmission of glutamate and increasing inhibitory synaptic transmission of GABA (Bacci et al., 2002) in brainstem areas such as the locus ceruleus. Therefore stimulation of Y1 receptors attenuates the somatic signs associated with nicotine withdrawal (Aydin et al., 2011; Rylkova et al., 2008).

Table 2: Novel Therapeutic Targets/Drugs Being Explored in Nicotine dependence in Randomized, Double Blind, Placebo-Controlled Clinical Trials and their Associated Interventions {Reference: http://clinicaltrials.gov.}

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Therapeutic Target</th>
<th>Phase</th>
<th>Study title</th>
<th>ClinicalTrials.gov Identifier:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil</td>
<td>Modulation of dopaminergic neurotransmission</td>
<td>Phase 3</td>
<td>Studying the Effects of Administration of Polyunsaturated Fatty Acids (PUFAS) of Omega-3 Series in Nicotine dependence</td>
<td>NCT01735279</td>
</tr>
<tr>
<td>RS1051730</td>
<td>Nicotinic acetylcholine receptor gene</td>
<td>Not provided</td>
<td>Smokers Response to nicotine dependence Genotyping</td>
<td>NCT01780038</td>
</tr>
<tr>
<td>EVP-6124</td>
<td>Nicotinic Acetylcholine Receptor agonist</td>
<td>Phase 2</td>
<td>A Safety and Cognitive Function Study of EVP-6124 Versus Placebo in</td>
<td>NCT01480232</td>
</tr>
<tr>
<td>Treatment</td>
<td>Mechanism</td>
<td>Phase</td>
<td>Description</td>
<td>Trial Identifier</td>
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<tr>
<td>--------------------------</td>
<td>------------------------------------</td>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Nicotine Patch</td>
<td>Nicotine acetylcholine receptor Agonist</td>
<td>Phase 4</td>
<td>Nicotine Patch for Nicotine Dependence in Individuals With Schizophrenia or Schizoaffective Disorder - 1</td>
<td>NCT00046813</td>
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<td>Nicoderm CQ</td>
<td>Nicotine acetylcholine receptor</td>
<td>Phase 2</td>
<td>Assessment of High Dose Transdermal nicotine for Fast Metabolizers of Nicotine</td>
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<td>Varenicline</td>
<td>Nicotinic receptor partial agonist</td>
<td>Phase 3</td>
<td>Pharmacogenetics of Nicotine Addiction Treatment</td>
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<td>Bupropion</td>
<td>Dopaminergic receptor inhibitor</td>
<td>Phase 4</td>
<td>Bupropion for ADHD in Adolescents With Substance Use Disorder</td>
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<td>Naltrexone</td>
<td>Opioid receptor antagonist</td>
<td>Phase 2</td>
<td>Targeted Interventions for Weight-Concerned Smokers</td>
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<tr>
<td>Chantix (Vareniclin)</td>
<td>Nicotinic receptor partial agonist</td>
<td>Phase 2</td>
<td>Combination Bupropion / Varenicline for Smoking Cessation in Male Smoker</td>
<td>NCT01806779</td>
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<tr>
<td>Fluoxetine</td>
<td>Selective serotonin reuptake inhibitor (SSRI)</td>
<td>Phase 3</td>
<td>Fluoxetine as a Quit Smoking Aid for Depression-Prone Smokers</td>
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<td>Baclofen</td>
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<td>Baclofen Effects in Cigarette Smokers</td>
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<td>Zyban</td>
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<td>Phase 2</td>
<td>Gemfibrozil for Nicotine Dependence</td>
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<td>Description</td>
<td>Phase</td>
<td>Study</td>
<td>NCT Number</td>
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<td>Pioglitazone</td>
<td>Nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ)</td>
<td>Phase 1, Phase 2</td>
<td>Pioglitazone for Heroin and for Nicotine Dependence</td>
<td>NCT01395797</td>
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<td>Selegiline</td>
<td>Selective irreversible MAO-B inhibitor</td>
<td>Phase 2</td>
<td>Selegiline Patch for Treatment of Nicotine Dependence</td>
<td>NCT01330030</td>
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<td>Propranolol</td>
<td>Non-selective beta blocker</td>
<td>Phase 3</td>
<td>Memory Reconsolidation Blockade as a Novel Intervention for Nicotine Dependence</td>
<td>NCT00916721</td>
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<td>Ondansetron</td>
<td>5-HT₃ receptor antagonists</td>
<td>Phase 2</td>
<td>Role of Metabolites in Nicotine Dependence (3) – 6</td>
<td>NCT00000289</td>
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<td>Cotinine fumarate</td>
<td>Desensitizes neuronal nicotinic acetylcholine receptors</td>
<td>Phase 2</td>
<td>Role of Metabolites in Nicotine Dependence (1) – 1</td>
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<td>Selective D₃ Antagonist</td>
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<td>Effectiveness of GSK598809, a Selective D₃ Antagonist</td>
<td>NCT01188967</td>
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<td>GW468816</td>
<td>NMDA Glycine Site Antagonist</td>
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<td>Effectiveness of GW468816, an NMDA Glycine Site Antagonist, for Prevention of Relapse to Smoking</td>
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2.12 NOVEL PHARMACOLOGICAL APPROACHES FOR NICOTINE WITHDRAWAL SYNDROME:

2.12.1 Src Kinases:

Src protein tyrosine kinase family is categorized into non-receptor tyrosine kinases and consists of nine members (Thomas and Brugge, 1997). Src, Fyn, Yes, and Yrk are ubiquitously expressed members of the family, whereas Blk, Fgr, Hck, Lck, and Lyn are expressed in more restricted patterns (Thomas and Brugge, 1997). Many of these src family kinases (cytoplasmic protein tyrosine kinases) are abundantly expressed in the central nervous system (Schlessinger, 2000). Recently, Src family kinases have been demonstrated to mediate continued nicotinic receptor activation based up-regulation of N-Methyl-D-Aspartic Acid (NMDA) receptors, which are in turn involved in the development of withdrawal syndrome in subjects having physiological dependence related to nicotine use (Rehni and Singh, 2013). Further, it has been shown that Src mediate the activation of extra-cellular receptor kinases during continuous nicotine treatment (Welsby et al., 2009). Moreover, src-kinase activation is reported to mediate NF-κB transduction system, which is also mediating pathogenesis of substance dependence (Thompson et al., 2006). Therefore, src-kinase seems to be a potential target for controlling the biochemical course of nicotine dependence and withdrawal.

Thus, it is suggested that the activation of src-family-kinases might play a significant role in mediating the pathogenesis and progression of nicotine dependence and that the pharmacological manipulation of src-family-kinases exert an ameliorative effect on nicotine withdrawal induced abstinence syndrome.
2.12.2 Nuclear factor-kappa-B (NF-κB):

Nuclear factor kappa-light-chain-enhancer of activated B cells is found in all animal cell types, a protein complex that controls the transcription of DNA. NF-κB is involved in immune and cellular responses. Dysregulation of NF-κB has been linked to various inflammatory and autoimmune diseases. It is likewise responsible for cancer, septic shock, viral infection, and decrease immunity. It plays a prominent role in processes of memory and synaptic plasticity. All proteins of the NF-κB family share a homology domain in their N-terminus and RelA, RelB, and c-Rel domain in their C-terminus region. Large precursor unit’s p105 and p100 are synthesized from NF-κB1 and NF-κB2 proteins, which undergo processing to generate the mature NF-κB subunits, p50 and p52 subunits, which is mediated by the ubiquitin/proteasome pathway. NF-κB dimers exist in a latent form in the cytoplasm bound by the IκB inhibitory proteins. NF-κB-inducing stimuli activate the IκB kinase complex (Karin et al., 2004) that phosphorylates IκB, leading to its ubiquitination and subsequent degradation in the canonical NF-κB activation pathway. IκB degradation exposes the DNA-binding domain and nuclear localization sequence of NF-κB and also permits its stable translocation to the nucleus along with the regulation of target genes. A prominent role for NF-κB transcription factors has been demonstrated throughout the immune system, where NF-κB-regulated gene expression is essential for processes of inflammation and host defense (Li and Verma, 2002). NF-κB
transcription factors are expressed throughout the central nervous system in both in neurons and in non-neuronal cells, such as glia and Schwann cells. Further, Nuclear factor-kappa-B is a transcription factor, which causes the activation of various transduction signals or multiple downstream signals to the nucleus. These signals resulting in the regulation of a number of Nuclear factor-kappa-B dependent genes responsible for the transcription of various inflammatory mediators like cytokines, which are in turn drawn in mediating addictive effects of nicotine on the central nervous system (Chen et al., 2006). NF-κB is a crucial regulator of many physiological and patho-physiological processes based on neuronal excitability (Baueerle, 1991). Nuclear factor-kappa-B has been reported to be transcribed in various part of the brain cells (O’Neill and Kaltschmidt, 1997). The literature has shown that exposure to nicotine increases the activation state of the NF-κB signaling pathway. Further, NF-κB is also suggested to facilitate the development of the rewarding aspects of chronic nicotine treatment in rodents (Dennis et al., 2005). Thus, nuclear factor kappa B activation has been proposed to be an important target mediating the progression of the nicotine withdrawal syndrome.

**Figure 7:** The *Nuclear factor-kappa-B* signaling module mediates wide variety extracellular and intracellular signals to control a diverse set of cellular responses.
2.12.3 C-C chemokine receptor type 2 (CCR 2):

CCR2 is a protein that in humans is encoded by the CCR2 gene that encodes for monocyte chemoattractant protein-1 (CCL2), a chemokine which specifically mediates monocyte chemotaxis in various inflammatory diseases such as rheumatoid arthritis and tumors by monocyte infiltration. C-C chemokine receptor type 2 mediates agonist-dependent calcium mobilization and inhibition of adenyl cyclase (El Khoury et al., 2007). Recently, NF-κB activation has been reported to control the transcription and biochemical activation of chemokines and thus regulate inflammatory processes, which are, in turn, proposed to precipitate withdrawal syndrome in nicotine (Palma-Nicolas et al., 2010). Prolonged nicotine treatment has been shown to enhance the transcription of chemokines and their respective receptors in the brain cells (Pace et al., 2011). Chemokine C-C motif ligand 2 (CCL2) is a potent chemotactic cytokine protein that is released from nicotine treated neurons in the brain (Bradford et al., 2011). Moreover, CCR2 activation has been implicated in the development of opioid abuse related human immunodeficiency virus-1 neuropathogenesis (El-Hage et al., 2006). However, the effect of pharmacological modulation of NF-κB activation linked chemokine activation on the nicotine withdrawal syndrome has not been examined.

2.12.4 Farnesyltransferase subtype I:

Protein prenylation implicates the transfer of a farnesyl moiety to C-terminal cysteine(s) of the target protein (Casey and Seabra, 1996). Prenylation is a post-translational lipid modification involving covalent addition of geranylgeranyl (20 carbon) moiety derived from mevalonic acid to conserved cysteine residues at or near the C-terminus of proteins that is catalyzed by protein farnesyltransferase I (Elnav and Glenn, 2003). There are basically three main classes of prenyltransferases: Isoprenyl pyrophosphate synthases (IPPSs), Protein prenyltransferases and Prenyltransferases (Liang et al., 2002). Farnesyl transferase (FT) is responsible for posttranslational lipidation (Casey and Seabra, 1996). In that respect are three enzymes that carry out prenylation in the cell, (a) Farnesyl Transferase (b) Caax protease (c) Geranylgeranyl Transferase I (Reid et al., 2004).
Farnesylation mediates protein-protein and protein-membrane interactions that are involved in various neurodegenerative and inflammatory disorders (Rehni and Singh, 2013). Farnesyltransferase (FTase) adds a farnesyl group (15-carbon isoprenoid) to target proteins, which is vital for cellular signaling (Eastman et al., 2006). Farnesyltransferase posttranslationally modifies various proteins by adding a farnesyl group with the help of thioether linkage to the -SH of the cysteine moiety of target proteins. This process, is known as farnesylation (Lane and Beese, 2006), which creates a hydrophobic binding pocket for farnesyl diphosphate (Furfine et al., 1995; Micali et al., 2001). FTIs were primarily developed as anticancer agents that blocking protein farnesylation by binding of activated Ras proteins to the plasma membrane and thereby block the constitutive signaling activity that provokes cell division (Cox et al., 1992; Long et al., 2002; Agrawal and Somani, 2009; Gordon et al., 2012). Farnesylfarnesylation facilitates membrane anchoring and is considered essential for the sub-cellular targeting and activation of RHO family proteins (Hori et al., 1991; Solski et al., 2002). Farnesylfarnesylation may also be important for protein–protein interactions, such as the binding of RHO proteins to RHO GTPase activating proteins (RHO-GAPs), which stimulate GTP hydrolysis and inactivation. Previous reports showed that nicotinic receptor stimulation leads to activation of the Rho family of small G-proteins that would lead to activation of Rho kinase (Kimura et al., 1996). It has been demonstrated that excitatory receptor agonist-induced Rho activation is Ca\(^{2+}\) dependent (Wang et al., 2006). Thus, inhibiting the farnesylfarnesylation of RHO family proteins might interfere with their targeting to membranes and their function to attenuate the pathogenesis of nicotine dependence. Farnesyltransferase I is responsible for lipid modification of several signaling proteins, such as Rho family small GTPase Rac1, which is involved in modulating neuronal physiology (Wu et al., 2010), by enhancing cyclooxygenase expression and generation of reactive oxygen species which are known to mediate nicotine withdrawal (Ghavami et al., 2012). Farnesyltransferase inhibitors stops tumor growth and metastasis, mediated by RHO family members (Sahai and Marshall, 2002; Caraglia et al., 2005; Sjogren et al., 2007). RHO family proteins play a salient part in migration of macrophages and lymphocytes into tissues, due to inflammatory
stimuli, and trigger NF-κB signaling, reactive oxygen species production, phagocytosis and cytokine production (Heasman and Ridley, 2008) that are in turn described to be involved in nicotine addiction. Therefore, inhibiting FTase-I have been reckoned as a possible strategy to inhibit the proinflammatory activities of RHO family proteins and to treat cancer, autoimmune and inflammatory inflammatory (Connor et al., 2006; Nagashima et al., 2006). The action of these proteins (e.g., Ras and Rho) are inhibited by prenylation inhibitors i.e farnesyltransferase subtype I inhibitors that block their isoprenylation (Graham et al., 1998). Because of this, blocking prenylation of Rho and Ras GTPases by farnesyltransferase type I inhibitor might serve as a possible strategy to preclude the maturation of the nicotine withdrawal syndrome. Inhibiting the farnesylfarnesylation of RHO family proteins has also been suggested to explain the anti-inflammatory properties (Jain and Ridker, 2005; Greenwood et al., 2006).

Thus, inhibiting protein farnesylfarnesylation might be an approach to treat various inflammatory diseases, an important molecular event associated with the development of the nicotine withdrawal syndrome. Thus, it is suggested that the activation of protein farnesyltransferase type I might play an important role in mediating the progression of nicotine dependence and that the pharmacological manipulation of these proteins exert an ameliorative effect on nicotine withdrawal induced abstinence syndrome.

2.12.5 G protein-coupled receptor kinase 5:

G protein–coupled receptors (GPCRs) are seven-transmembrane domain receptors that found mostly in all eukaryotes and animals, responsible for several cellular responses (Filmore, 2004; Darke et al., 2006). The ligands that bind and activate these receptors include proteins, peptides, hormones, and neurotransmitters (Overington et al., 2006). GPCRs are involved in various neurodegenerative and inflammatory diseases (Overington et al., 2006). G protein–coupled receptors are activated by modulation of ion channels, cAMP signal pathway and the phosphatidylinositol signal pathway (Bjarnadottir et al., 2006). Activated GPCR,s causes a conformational change, which allows it to act as a guanine nucleotide exchange factor, that activates an associated G protein by
exchanging its bound GDP for a GTP through α subunit, which can dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type (Gαs, Gαi/o, Gαq/11, Gα12/13) (King et al., 2003; Wu et al., 2012) which are involved in behavioral and mood regulation associated with opioid dependence (Rehni et al., 2012). GPCRs in the mammalian brain binds with different type of neurotransmitters, including serotonin, dopamine, GABA, and glutamate that plays a prominent role in nicotine addiction (Singh et al., 2013). GPCR also regulates the immune system activity by modulating the TLR-induced immune responses from T cells (Attwood and Findlay, 1994) and various inflammatory responses. G protein-coupled receptor kinase 5 is an enzyme that encodes for guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family. Previous studies shown that GRK 5 proteins could trigger the activation of the β-arrestin 2 and ERK1/2 signaling pathway that triggers the desensitization of GPCRs which plays a prominent role in drug dependence (Kunapuli and Benovic, 1993; Franklin and carrasco, 2013). Previous studies concluded that continuous administration of CP55940 increases CB2 receptor phosphorylation, selectively up regulates the level of GRK5 mRNA and enhances the protein expression in rat PFCx. CP55940 also decreased the levels of GRK2 mRNA and protein in limbic brain and in cultured neuronal cells treated with morphine (Franklin and carrasco, 2013). Opiate addiction: In rats treated chronically with morphine, GRK2 levels increased in the locus coeruleus. This increased GRK2 activity may both compensate for hyperstimulation of central nervous system opioid receptors and contribute to the problem of opiate tolerance and dependence (Wu et al., 2012). Furthermore, GRK is known to regulate the signal transduction cascade of dopamine receptor-GPCR complex as well as their desensitization based uncoupling (Cho et al., 2010). GRK-5 mRNA have been noted to be highly expressed in various parts of brain which are documented to mediate the precipitation of nicotine withdrawal syndrome viz. brain septum, cingulate cortex, septohippocampal nucleus, anterior thalamic nuclei, medial habenula, and locus coeruleus (Erdtmann-Vourliotis et al., 2001; Balfour, 2004) and have recently been shown to regulate GPCR mediated regulation of nicotinic
acetylcholine receptor signaling (Bibevski et al., 2000; Liu et al., 2000). Therefore, G-protein coupled receptor kinase-5 seems to be a potential target for controlling the biochemical course of nicotine dependence and withdrawal. Thus, it is concluded from the above discussion that the aggravation of GRK 5 kinase might play an essential role in mediating the pathogenesis and succession of nicotine dependence and that the pharmacological modulation of GRK 5 kinase exert an ameliorative effect on nicotine withdrawal induced abstinence syndrome.

**Figure 8:** Cell Biology of G-protein-coupled receptors-5 in Drug Dependence: desensitization, internalization and recycling of G-protein-coupled receptors are intimately connected (Breitwieser, 2004).

2.13. Pharmacological Interventions Employed in the Present Study:

2.13.1 **SU-6656:**

A study of Blake et al., (2000) has shown that SU6656 is a selective inhibitor for the Src family of tyrosine kinases.

2.13.2 **Ammonium pyrrolidine dithiocarbamate:**

Ammonium pyrrolidine dithiocarbamate (APD) is a selective inhibitor of NF-κB (Schreck et al., 1992).
2.13.3 **RS 102895:**

RS 102895 is a selective CCR-2 chemokine receptor antagonist (Mirzadegan et al., 2000; Onuffer and Horuk, 2002).

2.13.4 **Ro 32-0432 Hydrochloride:**

Ro 32-0432 Hydrochloride, is a selective GRK-5 receptor (G protein-coupled receptor kinase-5 enzyme) Inhibitor (Moore et al. 1998).

2.13.5 **FTI-276 Trifluoroacetate:**

FTI-276 Trifluoroacetate is a selective inhibitor of Farnesyltransferase subtype I (Lerner et al., 1995).

![Diagram](image)

**Figure 9:** SU-6656, Ammonium pyrrolidine dithiocarbamate, RS 102895, Ro 32-0432 Hydrochloride, FTI-276 Trifluoroacetate appears to be a promising target in smoking cessation.