Nicotine dependence has been characterized by both physiological adaptations (e.g., tolerance and withdrawal) and other accommodating behaviors (e.g., time spent in activities necessary to obtain/use nicotine and recover from its effects and the forfeiting or reduction of important social, occupational or recreational activities) (Zhan et al., 2012). Adults who smoke cigarettes develop nicotine dependence induced withdrawal syndrome, which is highly comorbid with anxiety and mood disorders (DiFranza et al., 2000; Zimmerman et al., 2002; John et al., 2004). Nicotine dependence is a diagnosable mental disorder according to DSM-IV (American Psychiatric Association, 2000; Diagnostic and Statistical Manual of Mental Disorders, 1994). Various preclinical and clinical studies have revealed that nicotine produces tolerance and leads to physical dependence (Naha et al., 2009; Vançelik et al., 2009). Withdrawal from chronic use of nicotine results in an abstinence syndrome, which includes increased nicotine craving, anxiety and pain sensitivity, restlessness, appetite, and decreased cognitive capabilities (Le Foll and Goldberg, 2009; Portugal and Gould, 2009). The withdrawal syndrome is considered one of the major causes of the high relapse rate among people undergoing smoking cessation (Le Foll and Goldberg, 2009). Therefore, research is being carried out in order to find out pharmacological approaches that might ameliorate the problem of nicotine dependence. The pharmaco-therapies that have recently been approved by the United States food and drug administration include the administration of following for the treatment of nicotine dependence nicotine replacement therapy [using nicotine gum, nicotine nasal spray, and transdermal nicotine (Berlin et al., 2012)] and administration of drugs like varenicline (Sofuoglu et al., 2009) and bupropion (Berrettini and Lerman, 2005). These pharmacological treatments available for smoking cessation are associated with symptomatic relief, but with low efficacy and more side effects. Moreover, the existing treatment options fail to inhibit the pathophysiological progression of the nicotine dependence. Therefore, the development of new strategies and treatments is necessary for attenuation of nicotine withdrawal syndrome associated with nicotine addiction (Zaparoli and Galduróz, 2012). However, none of the available options promises to conclusively treat the condition of nicotine dependence and its related abstinence syndrome.
Nicotine is a naturally occurring alkaloid found mainly in the members of the solanaceous plant family such as potato, tomato, green pepper and tobacco. Nicotine acts as an agonist for nicotinic acetylcholine receptors (nAChRs) present in central nervous system and peripheral nervous system. 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 and 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 α 7-subunits to form nAChRs with distinct pharmacological and pharmacokinetic properties. Acetylcholine acts as an endogenous neurotransmitter that binds and activates nicotinic acetylcholine receptors.

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; Alajaji et al., 2013).

These behavioral abnormalities develop gradually and progressively due to chronic and prolonged exposure of nicotine and other drugs of abuse. These can stay for months or years after discontinuation of drug use. As a consequence, drug addiction can be considered a kind of drug-induced neural plasticity (Nestler, 1993). The stability of the behavioral abnormalities that define addiction suggests a role for gene expression in this process. Agreeing to this view repeated and chronic exposure of nicotine alters the types and amounts of genes expressed in specific brain areas. 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, 1993; Hyman and Malenka, 2001; Nestler, 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, 2001a; Mackler et al., 2003). Consequently, various neurotransmitters are presumed to be affected by the reinforcing and rewarding effects of drugs of abuse like nicotine. Neuroadaptations that comes in response to chronic nicotine exposure, results in rise of dependence and withdrawal reactions associated with it.

Numbers of mechanisms have been proposed to mediate the neurobiology of nicotine dependence. Recently, George et al. (2007) has reported that precipitation of nicotine withdrawal in rats is principally modulated by an increase in the activation of corticotrophin releasing factor (CRF)-CRF₁ receptor system in the central nucleus of the amygdale. Farther, the CRF-CRF₁ system is likewise recognized to mediate the biology and conduct linked with negative reinforcement (i.e., setting off the unpleasantness of cessation) and relapse (i.e., a return to self-administration) also usually attributed to nicotine addiction (Dautzenberg et al., 1999). A recent report by Yuan et al. (2010) has proposed a mechanistic link between src-kinase activation and CRF system. Therefore, src-kinase might be linked with the overactivation of CRF--CRF₁ system which is mediating nicotine dependence and withdrawal.
A high-density genome wide association study for nicotine dependence conducted by Bierut and colleagues, (2007) has demonstrated that the principal genetic associations for nicotine addiction were in genes encoding for nicotinic receptor subunits. A number of candidate alleles at dopamine D2 receptor and δ opioid receptor genes have also been identified to cause nicotine dependence in clinical subjects (Berrettini and Lerman, 2005). Inspite of the recent advances in understanding the biochemical changes leading to nicotine dependence, the identification of an effective approach to control the pathophysiological progression of nicotine dependence remains an unresolved problem. Src-kinase has been reported to regulate the functions of neuronal nicotinic acetylcholine receptors, which are in turn responsible for nicotine dependence (Wang et al., 2004). Moreover, nicotine has been documented to induce β-arrestin–mediated activation of Src-Rb–Raf-1 pathway, thus indicating a causal link between the pharmacodynamic effects of nicotine and the activation of src linked transduction systems (Dasgupta et al., 2006). Nakao et al., (2009) and Barr et al., (2006) have confirmed that nicotine causes a transcriptional activation of NF-kappa B signaling complex, which is in turn known to work in tandem with src- kinases in mediating pathogenesis of substance dependence (Rehni et al., 2008; Rehni and Singh, 2011). Moreover, genetic transcription of src and src-kinases is reportedly associated with other transduction mechanisms involved in mediating both biological effects of nicotine as well as nicotine dependence (Charpantier et al., 2005; Courter et al., 2005; Dasgupta et al., 2006; Li et al., 2007; Li and Shang, 2007; Welsby et al., 2009). Moreover, src-kinase activation is reported to mediate NF-κB transduction system (Thompson et al., 2006). Therefore, src-kinase seems to be a potential target for controlling the biochemical course of nicotine dependence and withdrawal.

Moreover, dopamine and CRF1 receptor system is known to modulate Gs mediated activation of adenylyl cyclase (AC)-cyclic adenosine mono phosphate (cAMP) pathway which is in turn known to mediate nicotine withdrawal syndrome (Mifsud et al., 1989; Hauger & Dautzenberg, 1999a; 1999b; Ikemoto & Panksepp, 1999; Dautzenberg et al., 2001; Balfour, 2004; Pluzarev and Pandey, 2004). Continued over activation of dopamine and CRF1-receptors and their
associated GPCR receptor systems leads to rapid G-protein coupled receptor kinase dependent phosphorylation of G\(_{\alpha s}\) subunit of G-proteins which further leads to uncoupling of GPCRs from the receptor systems which has been proposed to mediate the progression of nicotine dependence related withdrawal syndrome (Dautzenberg et al., 2001; Pluzarev and Pandey, 2004; Teli et al., 2005; Hauger et al., 2009). Moreover, differential G-protein coupled receptor kinase (GRK) expression in brain neurons is noted to regulate the dopamine, CRF1 and CRF2 receptor signal transduction cascades (Teli et al., 2005; Hauger et al., 2009). Further, chronic activation based uncoupling of CRF1 receptor from its GPCR is noted to attenuate the CRF’s capacity to regulate dopamine release in the nuclei accumbens (Bruijnzeeel et al., 2012; Lemos et al., 2012), another biochemical event important in the pathogenesis of nicotine withdrawal syndrome (Mifsud et al., 1989; Ikemoto & Panksepp, 1999; Balfour, 2004). Furthermore, GRK is known to regulate the signal transduction cascade of dopamine receptor-GPCR complex as well as their desensitization based uncoupling (Agnati et al., 2003; 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 signalling (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.

Nicotinic acetylcholine receptors (nAChRs) are fast-acting, ligand-gated cation channels which mediate Ca\(_{\text{2+}}\) influx based modulation of neurotransmitter release and synaptic plasticity, a phenomenon underlying the pathophysiological course of nicotine dependence (Wonnacott, 1997; Dajas-Bailador & Wonnacott, 2004; Fucile, 2004). The nicotinic acetylcholine receptors (nAChRs) are "cysteine-loop" ligand-gated ion channels regulated by G-protein coupled receptor systems through prenylation of cysteine residues present on their \(\beta_3\alpha_2\) subunits (Poth et al., 1997; Bibevski et al., 2000; Miller et al., 2007; Pohanka, 2012).
Prenylation is a class of lipid modifications involving covalent addition of farnesyl (15-carbon) isoprenoids to cysteine residues present on the surface of target proteins having a C-terminal "CAAX" motifs, where C is cysteine, A is an aliphatic amino acid, and X at the C-terminus of the protein (Del Villar et al., 1996). Farnesyltransferase is an enzyme system responsible for causing protein prenylation (Reiss et al., 1990; Goldstein et al., 1991; Leung et al., 2006). In addition, chronic nicotine exposure stimulates nicotinic cholinergic receptors (nAChRs) that trigger calcium entry which, in turn, signal multiple biochemical events leading to nicotine dependence (Jackson & Damaj, 2009). This post-receptor signalling is, in part, mediated by small GTPase proteins that require prenylation for their activity (Reuveni et al., 2000). Further, pharmacological modulation of farnesyltransferase subtype I, an enzyme responsible for prenylation, is noted to inhibit the activity of these GTPase proteins (Graham et al., 1998; Sun et al., 1998). In turn, farnesyltransferases have been noted to be highly expressed in various parts of the brain (Yokoyama et al., 1991; Hooff et al., 2010). Furthermore, farnesyltransferase type I mediated prenylation of GTPases (Avidor-Reiss et al., 1996) is noted to facilitate inflammatory mechanisms which are in turn known to mediate the development of nicotine dependence induced withdrawal syndrome (Wilkes, 2006; Ross & Rosen, 2002; Thompson et al., 2006). Therefore, it may be put forth that farnesyltransferase type I mediated prenylation might participate in the pathogenesis of nicotine dependence. Thus, it may be proposed that farnesyltransferase type I might be a potential target for controlling the biochemical course of nicotine dependence induced withdrawal and the pharmacological modulation of farnesyltransferase type I might therefore exert a beneficial effect on the development of nicotine withdrawal syndrome.

Many novel cellular and molecular mechanisms underlying nicotine dependence are being tested. Microarray studies and genomic scan of nicotine dependent (ND) have shown the enhanced expression of nuclear factor kappa B (NF-κB) complex genes linked with nicotine exposure (Sullivan et al., 2004). Nicotine mediated activation of Akt-dependent NF-κB pathway alters various cellular processes that showed the detrimental effects of smoking in cancer patients (Tsurutani et al., 2005). Numerous studies carried out on the regulation of
gene expression and other determinants of the intracellular redox state suggested that inducible nuclear transcription factor (NF-κB) and its activator protein IκBα have been implicated and control the transcription of genes involved in immune response, inflammation, apoptosis, neurodegeneration, and tumorigenesis (Chen and Greene, 2004). NF-κB normally expressed in central nervous system, but plays a key role in neuronal plasticity, learning, memory consolidation, neuro protection, substance dependence and neurodegeneration (Kaltschmidt et al., 1994; Schneider et al., 1999). Recent studies suggest an important role of NF-κB on proliferation, migration, and differentiation of stem cells of nervous system (Widera et al., 2006). Cigarette smoking stimulates expression of NF-κB in neurons over a period of time (Manna et al., 2006). It is known that nicotine acts through nicotine acetyl choline receptors in the brain and is responsible for addictive nature (Campain, 2004).

It has been demonstrated that NMDA receptor antagonists block the development of tolerance, sensitization, physical dependence and withdrawal to nicotine without altering the acute effects of nicotine (Jain et al., 2008). Moreover, protracted activation of the NMDA-type receptors has been shown to induce nuclear translocation and resultant activation of NF-κB via a calcium influx sensitive pathway (Guerrini et al., 1995; Lilienbaum and Israel, 2003).

Functional NF-κB complexes are constitutively expressed in essentially all cell types in the nervous system, including neurons, astrocytes, microglia, and oligodendrocytes (O’Neill and Kaltschmidt, 1997). Prolonged nicotine use stimulates the excitatory glutamatergic transmission that modulates reward circuitries and positive reinforcing actions (Wolf et al., 2000; Harris et al., 2003), by increasing dopamine release within the mesolimbic system (Corrigall and Coen, 1989; Corrigall et al., 1992).

Initially, nicotine acts on presynaptic α7 nicotinic acetylcholine receptors (nAChRs) located on dopamine nerve cells in the ventral tegmental area (VTA) and pre-synaptic nerve terminals in the nucleus accumbens and increases their
firing rates due to enhanced dopamine release (Pidoplichko et al., 1997; Fu et al., 2000; Gentry et al., 2002).

Nicotine also acts at presynaptic α7 nAChRs located upon glutamatergic efferents track that arises within the prefrontal cortex (PFC) enhances glutamate release in the ventral tegmental area (Suaud-Chagny, 1992; Mansvelder and McGehee, 2000). This enhanced glutamate release and so acts as NMDA and non-NMDA receptor sites on post-synaptic dopamine neurons and increases their firing rate (Gensch et al., 1976).

Selective inhibition of glutamatergic transduction system, reduces dopamine release in the meso accumbens (Schilstrom et al., 1998), that attenuates nicotine induced rewarding effect in dependent subjects (Chiamulera et al., 2001; Paterson et al., 2003). Both the NMDA receptor activation induced increase in calcium influx as well as the resultant transcriptional increase in the factors of NF-κB family have been shown to be causative of a multitude of pathophysiological alterations in the brain activity as exemplified by conditions like stroke, epileptic seizures, traumatic brain injury and spinal cord injuries (Mattson and Camandola, 2001). NF-κB is an inducible transcription factor regulating a battery of inflammatory genes involved in the progression of various diseases (Baeuerle, 1991; Gensch et al., 1976). Therefore, it may be proposed that NF-κB over-activation might be playing a potential role in the pathogenesis of nicotine dependence. Our laboratory has reported a preliminary pharmacological data indicating the ameliorative effect of modulating NF-κB on morphine dependent mice (Capasso and Sorretino, 1997; Capasso, 2001; Rehni et al., 2008). Thus, nuclear factor kappa B activation has been proposed to mediate the progression of nicotine dependence related withdrawal syndrome.

Microarray analysis of human smoker brain and cultured cells exposed to nicotine showed significant changes in expression of genes related to immune or inflammatory responses, including chemokines and chemokine receptors.
Mazzone et al., 2010; Wang et al., 2011). Chronic nicotine exposure has been shown to enhance the expression of inflammatory mediators, cytokines (IL-1β, TNF-α and IL-18), chemokines, and adhesion molecules (ICAM-1, VCAM-1, and P-selectins) in the neurons, and also causes the release of various chemokines from glial cells astrocytes present in the central nervous system (Bradford et al., 2011; Crews & Vetreno, 2011; Berchtold et al., 2013). Plasma cytokine/chemokine monitoring in nicotine dependent subjects could improve the stratification of nicotine consumers seeking treatment and thus facilitate the application of appropriate interventions, including management of heightened risk of psychiatric co-morbidity (Hossain et al., 2009). CC chemokine receptor 2 linked inflammatory processes have been proposed to mediate the intensification of the effect of basic transduction mechanisms involved in mediating sub-cellular and biochemical changes occurring during the development of nicotine dependence (Pace et al., 2011).

Chemokines are chemotactic cytokines that direct the progression of inflammatory processes by mediating the migration of cells that express the appropriate chemokine receptors and have been reported to be abundantly expressed in the various cell types of the central nervous system (Cuenca-López et al., 2010). Substantial expression of CCR2 chemokine has been demonstrated in the central nervous system (Banisadr et al., 2005), and has further been forwarded as an important mediator of nicotine dependence. Chemotactic cytokines play a pivotal role in the inflammatory process associated with smoking, which mediated via cysteine-cysteine motif chemokine receptor-2 (CCR-2) receptor (Hartung et al., 2008). Moreover this Nicotinic receptor mediated chemokine activation is also reportedly associated the nicotine induced pain hypersensitivity (White & Wilson, 2008). Such data could potentially contribute to the understanding of this component of the neuroinflammatory response following the development of nicotine dependence.
This study focused on the role CCR2 receptors on the development of nicotine dependence induced withdrawal syndrome in mice. Our laboratory has reported a preliminary pharmacological data indicating the ameliorative effect of modulating CCR-2 chemokine receptor on morphine dependent mice (Rehni & Singh, 2012). Thus, CCR-2 chemokine receptor activation has been proposed to mediate the progression of nicotine dependence related withdrawal syndrome.

Therefore, the present study has been designed to investigate the effect of RS 102895 hydrochloride, a highly selective CCR-2 chemokine receptor antagonist (Mirzadegan et al., 2000; Onuffer & Horuk, 2002) on the development of nicotine dependence induced withdrawal syndrome in mice.

Studies have shown the effects of chronic intermittent systemic nicotine exposure on the development of bio-behavioural adaptations (Pauly et al., 1992). Therefore, in the present study, intermittent subcutaneous nicotine injections were used to elicit nicotine dependence in mice in line with earlier reports (Biala and Weglinska, 2005).

The present study was designed to identify novel molecular targets that might potentially be manipulated pharmacologically so as to attenuate the pathophysiological progression of nicotine dependence induced withdrawal syndrome in mice. Therefore the present study investigated the effect of SU-6656, a selective src-kinase inhibitor (Blake et al., 2000), Ro 32-0432 hydrochloride: A selective GRK-5 receptor (G protein-coupled receptor kinase-5 enzyme) Inhibitor, Ammonium pyrrolidine dithiocarbamate (APD), a selective nuclear factor kappa-B (NF-κB) inhibitor (Schreck et al., 1992), RS 102895, a selective CCR-2 chemokine receptor antagonist (Mirzadegan et al., 2000; Onuffer & Horuk, 2002), FTI-276 trifluoroacetate, a selective inhibitor of farnesyltransferase subtype I (Lerner et al., 1995) on propagation of nicotine dependence and resultant withdrawal signs in vivo.