In the present investigation, administration 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 (Moore et al. 1998), 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 and Horuk, 2002), FTI-276 trifluoroacetate, a selective inhibitor of farnesyltransferase subtype I (Lerner et al., 1995) demonstrated a significant dose dependent attenuation of mecamylamine induced withdrawal syndrome in nicotine dependent mice. Therefore, our data, for the first time, provides evidence that src-kinase, GRK-5 kinase, farnesyltransferase subtype I, nuclear factor kappa-B & CCR-2 chemokine receptor based pharmacological modulation plays an important role in the expression of somatic signs of nicotine withdrawal syndrome. Nevertheless, further studies are required to elucidate the involvement of these transduction systems in the nicotine dependence. On the basis of the above discussions, it may be concluded that the inhibition of src-kinase, GRK 5 receptors, nuclear factor kappa B, farnesyltransferase subtype I and chemokine CCR-2 receptor significantly (p<0.01) attenuates the development of Nicotine dependence as observed in the mecamylamine-induced precipitation of withdrawal symptoms in nicotine dependent mice. Therefore, our present studies corroborate with existing data (Damaj et al. 2003; Biala and Weglinska 2005; Stoker et al. 2008) by showing that mecamylamine treatment in nicotine dependent mice elicits various somatic and non-somatic signs of nicotine withdrawal.

Nuclear transcription factor kappa B (NF-kappaB) an inducible transcription factor detected in nerve cells is found to be affected in many biological processes such as inflammation, natural immunity, development, apoptosis, antiapoptosis and also responsible for the recording of several cytokines and chemokine, which are in turn implicated in mediating various effects of nicotine on the cardinal neural system (Friedman et al., 2003; Hasgal et
Electro mobility shift analysis showed that chronic nicotine exposure activates inducible NF-kappaB by binding to the consensus sequence of DNA (Barr et al., 2006). Nicotine added to cell culture stimulates the degradation of IkappaB-alpha subunit that induces oxidative stress leading to activation of stress dependent NF-kappaB pathway in mesencephalic cells (Barr et al., 2006). The transcription factor NF-kB has diverse roles in the neural scheme, depending on the cellular context. NF-kB is constitutively activated in glutamatergic neurons, the transcription of which is maintained by the physiological basal synaptic transmission. In glia, NF-kB is inducible and regulates inflammatory processes that exacerbate diseases such as autoimmune encephalomyelitis, ischemia, and Alzheimer’s disease. Suppression of NF-kB in glee might ameliorate disease, whereas activation in neurons mediates certain vital neurological processes (Kaltschmidt and Kaltschmidt, 2009). Nicotine activation of NF-kB may be through binding to nicotine acetylcholine receptors. Earlier surveys have shown activation of NF-kB in the mouse brain stem, cerebellum, frontal cortex, hippocampus, and stratum following the long-term exposure of Nicotine (Manna et al., 2006).

Further NFkB activation has been reported to be associated with the upregulation of a number of inflammatory mediators, cytokines, neurotrophic factors, arachidonic acid metabolites and neurotransmitters that play a prominent role in nicotine dependence (Karin, 1995; Rao & Wu, 2000; Gensch et al., 2004; Kuperstein et al., 2008; Archer et al., 2012; Shi et al., 2013). Moreover, the stimulation of NF-kB linked signal transduction system has also been shown to modulate the activity of src-kinase (Rehni et al., 2012) and NMDA receptors (Zhao- Shea et al., 2013), which are further involved in mediating various pathophysiological alterations linked sub-chronic and chronic nicotine administration. Therefore, these chemical messengers might be mediating the NF-kB based precipitation of nicotine dependence related withdrawal syndrome. In our previous study, we have demonstrated ameliorative effect of diethyl
Discussion

dithiocarbamic acid, a relatively selective NF-κB inhibitor, on opioid withdrawal syndrome in mice (Rehni et al., 2008).

On the basis of the above discussion, it may be concluded that ammonium pyrrolidine dithiocarbamate, a selective NF-κB inhibitor, treatment attenuates the development of nicotine dependence as observed in the mecamylamine induced precipitation of withdrawal symptoms in nicotine dependent mice.

Chemokines are chemotactic cytokines that direct the progression of inflammatory processes by mediating the migration of cells that convey the appropriate chemokine receptors. Chemokine C-C motif ligand 2 (CCL2) is a potent attractant protein for inflammatory cells. The biological effects of CCL2 are mediated via interactions with its receptor, chemokine C-C motif receptor 2 (CCR2) which is a G protein-coupled receptor and regulates the migration and infiltration of monocytes, T-lymphocytes and natural killer cells to areas of inflammation (Matsushima et al., 1989; Allavena et al., 1994; Carr et al., 1994). In the cardinal nervous system, CCR2 expression has been demonstrated in endothelial cells, astrocytes, microglia and neurons (Boddeke et al., 1999). In addition, CCR2 expressing cells are discovered in multiple brain areas including the hippocampus (Banisadr et al., 2005) and the expression patterns of CCR2 have shown to be changed in several neurodegenerative disorders (Conant et al., 1998; Kelder et al., 1998; McManus et al., 1998; Wu et al. 2008; Sokolova et al., 2009).

Nicotine exposure altered the circulating levels of pro-inflammatory mediators. Further, nicotine can act as both chemokines and chemo attractants (Chernyavsky et al., 2004) therefore; the release of chemokines is reportedly associated with nicotinic receptor transduction systems (Martínez-García et al., 2010; Kiguchi et al., 2012). Nicotine treatment activates microglia and astrocytes in the nucleus accumbens (NAcc) to stimulate the synthesis of cytokines and chemokines, and this holds significant implications for addictive behavior (Wang et al., 2011; Moylan et al., 2013). Nicotinic acetylcholine receptor mediated nuclear factor kappa B activation based transduction system has been reported to
be linked with the control of transcription and biochemical activation of chemokines and thus regulate the multitude of inflammatory processes, which in turn is proposed to participate the progression of nicotine associated withdrawal syndrome (Stevenson et al., 2005; Pace et al., 2011). Therefore, chemokine receptor activation might participate in the precipitation of nicotine withdrawal syndrome. RS 102895 is a relatively selective CC chemokine receptor 2 antagonist so present study demonstrated that RS 102895 produced a significant dose dependent attenuation of the development of nicotine dependence and thereby reduces withdrawal signs in vivo in nicotine dependent mice.

Src family members are non-receptor protein tyrosine kinases (PTKs), which are expressed in the neuronal cells (Erpel and Courtneidge, 1995). Multiple members of this family are expressed in the neuronal tissue like Src, Fyn, Yes, Fgr, Lyn, Hck, Lck, Blk and Yrk (Dymecki et al., 1992; Torigoe et al., 1992; Corey et al., 1993; English et al., 1993; Sudol et al., 1993; Corey et al., 1994; Anderson and Jorgensen, 1995; Appleby et al., 1995; Corey and Anderson, 1999). They participate in a variety of cellular processes like cytoskeletal assembly and organization, cell-cell contact, cell-matrix adhesion, induction of DNA synthesis, cell survival, and cellular proliferation (Erpel and Courtneidge, 1995). Further, Src PTK has been shown to be regulated by its posttranslational phosphorylation and dephosphorylation (Oda et al., 1999). Moreover, src-kinase activation has been implicated in the pathophysiological progression of certain brain disorders like intra-cerebral hemorrhage, seizures and ischemic neuronal injury (Sharp et al., 2008; Balosso et al., 2008; Wennn et al., 2008; Du et al., 2009). Furthermore, src-protein tyrosine kinases have been implicated in cocaine dependence via NMDA receptor activation linked mechanism (Schumann et al., 2009). Furthermore, studies have shown that nicotine activates inducible NF-kappa B (Yoshikawa et al., 2006), which has further been proposed to be involved in mediating substance dependence (Rehni et al., 2008). It is further noted that src-kinase activation mediates the stimulation of NF-κB transduction system (Thompson et al., 2006). Therefore, it was postulated that pharmacological
manipulation of src-kinase might exert a beneficial effect on nicotine dependent mice. This hypothesis of possible involvement of src-kinase activation in nicotine dependence is supported by our present study in which SU-6656, a selective src-kinase inhibitor (Blake et al., 2000), was found to dose-dependently attenuate the behavioral manifestations of nicotine withdrawal in nicotine dependent mice. Thus, it may be deduced that the activation of src-kinase is playing an important role in mediating the precipitation of nicotine withdrawal syndrome. However, the effect of src-kinase inhibition on nicotine withdrawal induced motivational aspects of behavior such as conditioned place aversion and impaired function of the mesolimbic dopamine system is yet to be evaluated in order to define the extent to which src-kinase activation mediates nicotine withdrawal syndrome. Moreover, detailed dissection of the transduction system(s) following the src-kinase activation that is leading to the abstinence syndrome also requires further experimental assessment. However, src kinases are regulated by ubiquitin-mediated proteasome enzyme system (Oda et al., 1999). Further, src-kinase activation has also been reported to be associated with a Src-Shc-Grb2-Ras-MAP kinase pathway (Korade-Mirnics and Corey, 2000). Therefore, these biochemical mechanisms may be participating in the pathophysiological progression of nicotine dependence. However, such a hypothesis needs a critical evaluation and a close experimental validation before its application into the clinical setting.

Although nicotine is known to activate src-kinase and downstream signaling effectors including NF-kB, the same effect is also known to be produced by other drugs capable of producing dependence like opioids. In this context, we have earlier shown that the activation of src-kinase pathway is involved in mediating the development of morphine dependence and withdrawal (Rehni and Singh, 2011). It may be proposed that src-kinase activation might be acting as a common denominator for substance dependence and withdrawal, and this signaling pathway may not be specifically linked to the activation of nicotinic receptors only. This activation may stem from multiple neural pathways triggered by drugs and might involve the modulation of reward, motivational, emotional
and cognitive processes. Therefore, further experimental work to determine whether or not the inhibition of src-kinase also abolishes nicotine withdrawal syndrome precipitated by a non-nicotinic antagonist like naloxone (an opioid receptor antagonist) is required. Generation of such data would make a valuable addition in the literature for establishing the cause and effect relationship between src-kinase activation and nicotine withdrawal associated with the activation of nicotinic as well as opioid receptors. Mecamylamine, a widely used broad-spectrum neuronal nicotinic acetylcholine receptor antagonist, at doses employed in the present study is also known to modulate calcium channels and NMDA receptors in brain and is thus not essentially a selective nicotinic acetylcholine receptor blocker at the doses employed in the present study (Papke et al., 2001). However, the association of these mechanisms with mecamylamine based precipitation of withdrawal syndrome as well as mediation of SU-6656 induced suppression of the syndrome is yet unexplored and merits experimental evaluation. Moreover, a study is required to evaluate the role of pharmacological inhibition of src kinase on nicotine withdrawal syndrome in mice induced using a more selective neuronal nicotinic acetylcholine receptor antagonist than mecamylamine at the said dose with a view of extending the present data closer towards clinical consideration.

A group has postulated that CRF-CRF1 receptor system plays a critical role in mediating the precipitation of nicotine withdrawal which may be based on an altered src kinase activity (George et al., 2007). Moreover, Pauly et al (1992), have shown an important role of changing plasma corticosterone levels in mediating tolerance associated with chronic nicotine exposure elicited by intermittent injections in mice. However, a detailed experimental investigation is required to characterize the role of differential interaction between corticosterone and CRF-CRF1 receptor system and their biochemical cross talk with src-src-kinase system in the development of nicotine dependence and tolerance in an independent manner. Moreover, Pauly’s work (1992), when viewed in the perspective of the present data, implicates the requirement of evaluating the role
of src kinase and other proposed mechanisms for their respective contribution differentially towards nicotine withdrawal and nicotine tolerance. Research integrating these individual potential targets and understanding their relative importance for the two aspects may give a clearer picture of composite mechanisms worthy of future attention by the clinicians.

Therefore, based on the above data, it may be suggested that search-coins might be taken in the development of nicotine dependence related withdrawal syndrome and thus, may function as a viable pharmacological target to tackle the problem of nicotine dependence.

G protein-coupled receptor (GPCR) kinase (GRK) is a family of serine/threonine kinases. Protracted over activation of GPCRs is noted to induce the transcription of GRKs which subsequently catalyze the phosphorylation of numerous GPCRs with various vital functions (Ferguson et al., 1996; Penn et al., 2000). This GRK-induced GPCR phosphorylation leads to homologous desensitization of GPCRs and causes their resultant uncoupling from CRF₁ receptors (Dautzenberg et al., 2001; Pluzarev and Pandey, 2004; Teli et al., 2005; Hauger et al., 2009) and dopamine receptors (Cho et al., 2010; Lemos et al., 2012), which might in turn cause the precipitation of nicotine withdrawal syndrome (Ikemoto and Panksepp 1999; Mifsud et al., 1989; Balfour, 2004;). Moreover, differential GRK expression in brain neurons is noted to regulate the CRF₁ and CRF₂ receptor signal transduction cascades (Teli et al., 2005; Hauger et al., 2009). In addition, protracted stimulation of CRF associated CRF₁-receptor activation and resultant stimulation of GPCR related signal transduction pathways, which has been noted to be the principal cause of nicotine withdrawal syndrome, is shown to cause rapid G-protein coupled receptor kinase dependent phosphorylation of Gαs subunit of G-proteins which causes the uncoupling of GPCRs from CRF₁ receptor system which has been proposed to mediate the progression of nicotine dependence (Dautzenberg et al., 2001; Pluzarev and Pandey, 2004; Teli et al., 2005; Hauger et al., 2009). Further, persistent activation based uncoupling of CRF₁ receptor from
its GPCR is noted to attenuate the CRF’s regulatory influence on dopamine release in the nucleus accumbens (Lemos et al., 2012), another neurological process facilitating the pathogenesis of nicotine dependence, reinforcement and resultant withdrawal syndrome (Ikemoto and Panksepp, 1999; Balfour, 2004; Mifsud et al., 1989). Furthermore, GRK is known to regulate the signal transduction pathways associated with the activation of dopamine receptor-GPCR complex as well as their desensitization based uncoupling (Cho et al., 2010). In addition, a GRK-sensitive Gα protein pathway has been noted to facilitate Gαα and Gαβγ subunits activation based facilitation of nAChR mediated currents (Liu et al. 2000), which in turn participates in the development of nicotine dependence. G-protein based modulation of neuronal nAChRs has been attributed to the activation of their β3α2 subunits (Poth et al., 1997; Bibevski et al., 2000) which are in turn noted to be closely regulated by GRKs. Among the seven known subtypes of GRKs (GRK1-7), GRK2, GRK3, GRK5 and GRK6 are noted to be expressed in a wide range of tissues such as heart, brain, lung and placenta, while GRK1, GRK4 and GRK7 are expressed exclusively in retina and testis, respectively (Penn et al., 2000). The wide expression of GRK5 in the most rat brain regions (Arriza et al., 1992; Erdtmann-Vourliotis et al., 2001) suggests that these GRKs may play important roles in regulation of neuronal signal transduction. Further, 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, it may be proposed that GRK-5 based alterations in nicotine receptor signaling mechanism participates in the pathogenesis of nicotine dependence related biochemical changes in the neurons which might eventually lead to CRF-dopamine pathway modulation dependent precipitation of nicotine withdrawal syndrome. This
contention is further supported by our present investigation with Ro 32-0432 which markedly suppressed the intensity of the mecamylamine precipitated withdrawal syndrome in nicotine dependent mice. Therefore, it may be deduced that the activation of G-protein coupled receptor kinase-5 is involved in the precipitation nicotine withdrawal syndrome.

Abrupt nicotine abstinence is noted to decrease the natural action of the mesolimbic dopaminergic system and therefore decrease the dopamine levels in the nucleus accumbens in the dependent brain, therefore triggering the affective and somatic signs of withdrawal (Hildebrand et al., 1998; Rada et al., 2001). Anxiousness is a symptom of withdrawal and it plays as a potent negative reinforcer that promotes smoking (Brown et al., 2001). Also being a product of nicotine withdrawal, stress and anxiety can worsen the symptoms of withdrawal, which contributes to increased craving and relapse (Perkins and Grobe 1992; Carey et al., 1993). The central nucleus of the amygdala (CeA), together with the bed nucleus of the stria terminalis and the posterior shell of the NAcc is part of the “extended amygdala” (Koob, 2010). This circuit and the HPA axis play crucial roles in the processing of the negative affective states associated with the drug withdrawal (Koob and LeMoal, 2008; Koob and Volkow, 2010). Elevations in corticosterone and ACTH-releasing factor (CRF) are observed during acute withdrawal from nicotine (Koob and LeMoal, 2008). However, which particular aspects of the neurobiology of nicotine dependence are associated with GRK-5 activation based precipitation of nicotine withdrawal; need to be assessed in future works.

On the basis of the above discussion, it may be concluded that the inhibition of G-protein coupled receptor kinase-5 attenuates the propagation of nicotine dependence and thereby reduce withdrawal signs as observed in the mecamylamine-induced precipitation of withdrawal symptoms in nicotine dependent mice. Nevertheless, further studies are required to elucidate the
involvement of G-protein coupled receptor kinase-5 related transduction systems in the nicotine dependence.

Proteins that terminate with a carboxyl-terminal “CAAX” motif (C = cysteine, A = aliphatic amino acid, X = any amino acid), such as the Ras and Rho proteins, undergo farnesylation by protein farnesyltransferase (FTase) (GGTase I) (Casey and Seabra, 1996; Appels et al., 2005). Cysteine residues present on the transmembrane domains of nicotine receptors are noted to confer them the sensitivity to nicotine (Le Novère et al., 2002). Moreover, the nature of cysteine residues present on the G-proteins coupled to dopamine and CRF receptors are documented to regulate the effectiveness of information flow from the nicotine receptor to the G protein coupled receptor linked transduction pathway (Lan et al., 2009; Hauger et al., 2006; Ishibashi et al., 2009; Yu et al., 2010). Farnesyltransferase based prenylation of cysteine residues present on the G-protein α and βγ subunits and GTPases are necessary for membrane localization and for functional heterotrimeric G protein coupled receptor signaling (Kinsella and Maltese, 1992; Mulligan et al., 2010). Thus, it is evident that farnesylation based biochemical changes contribute towards the pharmacology of nicotine. Further, sustained activation of nicotine receptor and continued persistent stimulation of dopamine and CRF receptors are known to mediate the pathogenesis of nicotine dependence (Walton et al., 2001; Ishibashi et al., 2009; Camí and Farré, 2003). Furthermore, farnesylation of small GTPases, mediated by farnesyltransferase (Konstantinopoulos et al., 2007; Deraeve et al., 2012; Stubbs and Von Zee, 2012), are noted to initiate a positive feedback mechanism leading to the over-activation of the signal transduction cascades viz., the Raf/MEK/ERK (Extracellular Signal-Regulated Kinases) pathway, the MEKK/SEK/JNK (Jun N-terminal Kinases) pathway, a PI3K (Phosphatidylinositol 3-Kinase)/Akt/NF-KappaB (Nuclear Factor-Kappa B) pathway, a p120-GAP/p190-B/Rac/NF-KappaB pathway, and a Raf/MEKK1/IKK (I-KappaB Kinase)/I-KappaB/NF-KappaB pathway (Teramoto et al., 1996; Gay et al., 1999; Yeung et al., 2001; Roberts and Der, 2007; Konstantinopoulos et al., 2007; Steelman et al., 2011; Pace et al., 2011). These
mechanisms are known to bring about the activation of various inflammatory pathways associated with interleukin 1-β, interleukin-6, interleukin-10, interleukin-12, tumor necrosis factor-α, nitric oxide synthase, C-reactive protein, cyclooxygenase and prostaglandins which are in turn involved in mediating the pathogenesis of nicotine withdrawal syndrome (Heimdal et al., 1999; Guo et al., 2001; Jain et al., 2008; Shin et al., 2008; Di Matteo et al., 2010; Pace et al., 2011; Díaz-Marsá et al., 2012; Nunes et al., 2012). Therefore, it may be proposed that farnesylation based alterations in the receptor signaling mechanisms participate in the pathogenesis of nicotine dependence.

Thus, it may be proposed that the withdrawal syndrome induced by sub-chronic nicotine administration followed by a mecamylamine injection may involve the activation of protein farnesylation. Farnesyltransferase are critical protein prenyltransferase enzyme systems that catalyze the process of protein farnesylation (Reiss et al., 1990; Goldstein et al., 1991; Leung et al., 2006). Further, a high level of transcription and enzymatic activity of alpha subunit of a heterodimeric protein farnesyltransferase has been noticed in the rat brain tissue, suggesting their active role in mediating neuronal functions (Chen et al., 1991; Joly et al., 1991). Therefore, pharmacological modulation of protein prenylation by inhibiting farnesyltransferase was hypothesized to possess a potential ameliorative effect on nicotine withdrawal syndrome. FTI-276 is a selective inhibitor of farnesyl transferase (Lerner et al., 1995). In the present investigation, the administration of FTI-276 was observed to attenuate propagation of nicotine withdrawal syndrome in mice. It may thus be proposed that the ameliorative effect of FTI-276 might be ascribed to its inhibitory effect on protein farnesyltranferase. However, future investigations are required to validate the potential correlation between the farnesyltransferase inhibiting potential of FTI-276 and its nicotine withdrawal suppressing effect of FTI-276.

Various signal transduction systems are reported to be associated with the activation of farnesylation (McCarty, 2003; Lazzerini et al., 2007; Zhuravliova et
Therefore, it may be put forth that the signal transduction systems linked to farnesyltransferase might be playing a potentially significant role in causing the development of nicotine withdrawal syndrome. However, these views need to be substantiated by following a rigorous experimental methodology before such a concept is considered for its clinical utility. Moreover, the effect of farnesyltransferase inhibition on nicotine withdrawal induced motivational aspects of behavior such as conditioned place aversion and impaired function of the mesolimbic dopamine system is yet to be evaluated in order to define the extent to which farnesyltransferase activation mediates nicotine withdrawal syndrome.

Therefore, based on the above data, it may be suggested that src-kinase, GRK-5 kinase, farnesyltransferase subtype I, nuclear factor kappa-B & CCR-2 chemokine receptor might be involved in the development of nicotine dependence related withdrawal syndrome and thus, may serve as a viable pharmacological targets to tackle the problem of nicotine addiction.