6. DISCUSSION

Depression is an important contributor to the global burden of the mental diseases affecting people in all the communities across the world. Today, depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression in the previous year\textsuperscript{100,101}. Currently available antidepressants used to treat major depressive disorder (MDD) unfortunately take weeks to months to achieve their therapeutic beneficial effects, commonly resulting in considerable morbidity and increased risk of suicidal behaviour. Our lack of understanding of the precise cellular dysfunction in mental illness and of the mechanism of action of existing effective pharmacological treatments is one of the reasons that therapies with a more rapid onset of antidepressant action have not been developed\textsuperscript{102}.

Major depression is treated with drugs that inhibit the reuptake and metabolism of biogenic amines. Drugs like TCA are still used and in experimental studies, they are used as standard against which study drugs are compared. However, these agents do not exhibit either a faster onset of action or greater efficacy than their predecessors\textsuperscript{103}. Therefore, there are concerted efforts underway to develop antidepressants with possible advantages over currently used antidepressant drugs\textsuperscript{104}.

On reviewing the literature, there are controversial reports about antidepressant action of nicotine. Hence this study was designed to evaluate antidepressant action of nicotine by s.c. as well as inhalational route.

Depression is a heterogeneous disorder and some of its symptoms are difficult to be reproduced in laboratory animals. The question therefore remains, whether the animal is ‘depressed’ or not. However, it is not necessary for an ‘ideal’ animal model of depression to exhibit all the abnormalities of depression-relevant behaviours, just as the patients do not manifest every possible symptom of depression\textsuperscript{65}.

In this study, two models of depression are used to evaluate antidepressant activity of the study treatment. Isolation induced hyperactivity model produces social disturbances in animal and stress evoked i.e. learned helplessness model of depression fulfills validating criteria. Most models currently used rely on either actions of known antidepressants or responses to stress. An animal model of depression should produce similar pathophysiology and comparable etiology. It is expected that drugs effective in treating depression in humans should produce
positive effects in these models. Laura A. León (2008) suggested that due to precision in animal modeling, animal models like active avoidance, social isolation, learned helplessness, behavioral despair, genetic depression models would be important to evaluate the antidepressant activity of a new potential compound. Social isolation stress is a valuable stress paradigm for investigating the effects of chronic psychosocial stress on various pathophysiological alterations in animals.

Isolation induced hyperactivity model is basically related to theoretical aspect of depression and learned helplessness model was used to evaluate clinical utility of study treatment. Spontaneous locomotor activity is used to see CNS stimulant activity of nicotine, in normal rats.

In isolation induced hyperactivity model, the locomotor activity was increased after isolation. These results are consistent with previous reports.

It was found that imipramine i.e. standard antidepressant drug and nicotine by subcutaneous and inhalational route showed antidepressant activity by reducing locomotor activity in isolated rats. Similarly, in combination treatment, single dose nicotine(s.c.) with imipramine reduced locomotor activity and showed comparable antidepressant activity to imipramine alone. However, single dose nicotine(inhal.) combined with imipramine showed additive antidepressant activity compared to imipramine alone.

Likewise, seven days treatment of nicotine(s.c.) with imipramine showed comparable antidepressant activity with imipramine at all time points. However, there was an additive effect with nicotine(inhal.) combined with imipramine for seven days at all time points as compared to both imipramine and nicotine(inhal.) alone group.

In learned helplessness model, imipramine has shown antidepressant effect after 7 days administration. Nicotine administered subcutaneously and inhalational route showed positive escape response as compared to vehicle. However, nicotine(inhal.) showed increased better escape response as compared to imipramine.

Single dose of nicotine was combined with 7 days treatment of imipramine, nicotine by inhalation with imipramine depicted better antidepressant effect by showing increased positive escape response as compared to imipramine alone.
It was observed that, seven days combination of nicotine (s.c.) with imipramine showed comparable antidepressant effect to that of imipramine alone. Nevertheless, nicotine(inhal.) with imipramine showed additive effect compared to imipramine alone. Dopaminergic and serotonergic activity was increased in both nicotine(s.c.) and nicotine(inhal.) groups as compared with vehicle group.

Sedation score (central α2) diminished with nicotine (s.c.) and nicotine(inhal.). The results are more prominent with nicotine(inhal.) group. Thus, it can be inferred that nicotine acts by inhibiting central α2 receptors.

Nicotine(s.c.) and Nicotine(inhal.) failed to reduce duration of impaired righting reflex induced by propranolol via central β receptors.

To conclude therefore, antidepressant action nicotine is by increasing dopaminergic & serotonergic activity. Inhibiting α2 receptors centrally, it increases central NA activity which may contribute to its antidepressant action.

In estimation study, isolation induced hyperactivity model was used to induce depression in animal. neurotransmitters levels is prominently reduced in depressed animal.

In study treatment group, neurotransmitter levels is increased in imipramine, nicotine(s.c.) and nicotine(inhal.) group as compared to vehicle control group.

There is evidence to indicate that nicotinic acetylcholine receptors (nAChRs) are involved in major depression. As pointed by Markou et al. (1998), epidemiological findings suggest that smokers more often demonstrate depressive symptoms than nonsmokers, and that depressed patients are less likely to cease smoking. Depressed smokers are more dependent on smoking and smoking cessation in these subjects is often followed by a depressive episode. Smokers with history of major depressive episode are more likely to relapse than smokers with no history of depression. Finally, smokers with a history of major depressive episode are more likely to relapse than smokers with no history of depression. Furthermore, nicotine patches can improve the mood of depressed patients. It has been hypothesized that nicotine produces antidepressant effects, and that smokers ‘self-medicate’ the underlying depressive illness with nicotine, and/or depressive symptoms produced by nicotine withdrawal.

Another hypothesis that appears, contradictory to the first, is based on findings indicating that most clinically effective antidepressants antagonize nAChRs. The antidepressant drug,
bupropion, antagonizes neuronal nAChRs\textsuperscript{107}. It has been used as the treatment for smoking addiction\textsuperscript{108}.

However, according to P Popik (2003) has reported that nicotine alone was inactive in tail suspension model of depression, but it increased antidepressant action of imipramine in combination treatment. This facilitating action of nicotine was seen at doses that did not affect locomotor activity\textsuperscript{109}.

In this study, nicotine given by inhalation route increased antidepressant action of imipramine in single dose. However, single dose or 7 days treatment of nicotine failed to increased antidepressant action of nicotine in isolation induced hyperactivity model.

P Popik (2005) evaluated antidepressant activity in ‘apomorphine-induced hyperactivity’ model of depression\textsuperscript{22,110}. In this model, parameter is potentiation of apomorphine induced hyperactivity. They reported antidepressant activity with 2 weeks chronic treatment with imipramine and after 1 week of the treatment of combination of nicotine and imipramine. Similar results were observed with 1 week administration of nicotine alone\textsuperscript{22}.

Tizabi(1999) reported that, nicotine(0.4mg/kg) for 14 days potentiated the effect of antidepressant action of imipramine as well as selective serotonin reuptake inhibitors in forced swim test model of depression. He stated that this action of nicotine could be attributed to the stimulation of nicotinic cholinergic receptors at the beginning of therapy with antidepressants that can result in a decrease of the ß-adrenoceptors density\textsuperscript{111}.

However, preclinical data regarding antidepressant action on nicotine are ambiguous. Ferguson et al (2000) reported that nicotine did not influence the learned helplessness response, though a subtype selective nicotine acetylcholine receptor agonist produced antidepressant like effect.

Perhaps more suggestive are studies on the “Flinder’s Sensitive Line” rats, which are regarded as a “genetic animal model of depression”\textsuperscript{105}. In these rats, which demonstrate an exaggerated immobility in drug free state, acute and chronic administration of nicotine significantly improved performance in forced swimming test\textsuperscript{112}. The most convincing results demonstrating antidepressant like effect of nicotine were reported by Semba et al (1998) who showed that the chronic treatment with nicotine produced antidepressant like effect in the learned helplessness model of depression\textsuperscript{113}.

However, some authors report contradictory results. According to JT Andreasen (2009), nAChRs receptors antagonist mecamylamine (ganglionic blocker), dihydro-ß-erythroidine
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(α4β2-selective antagonist) and methyllycaconitine(α7-selective antagonist) showed antidepressant effect in tail suspension and forced swimming test\textsuperscript{114}.

Literature suggests involvement of nicotinic acetylcholine receptors (nAChRs) in major depression. However, it is controversial whether the antidepressant-like effect of nACHR modulation is induced by activation, desensitization or inhibition of central nACHRs. In addition, the specific nACHR subtypes involved remain unknown\textsuperscript{114}.

As can be seen from above reports, that preclinical and clinical data regarding antidepressant action of nicotine are ambiguous.

This study was therefore planned to evaluate antidepressant action of nicotine using two models. Isolation induced hyperactivity and learned helplessness models were used to study antidepressant activity of nicotine in rats.

In this study, we compared the antidepressant effects of imipramine, a standard antidepressant drug and nicotine administered by subcutaneous and inhalational routes.

The reports of subcutaneous nicotine have been inconsistent in various studies on depression, therefore we planned to study subcutaneous route in isolation induced hyperactivity and learned helplessness model of depression.

Smoking is the commonest route by which nicotine is inhaled, hence we have chosen the inhalational route to evaluate antidepressant action of nicotine.

Effect on study treatment on spontaneous locomotor activity in normal rats, standard antidepressant drug imipramine had no significant effect. However, nicotine by subcutaneous as well as inhalational routes increased locomotor activity, which may be because of its CNS stimulant action.

In isolation induced hyperactivity study, it was observed that locomotor activity decreased significantly at 10 min after inhalational nicotine in both normal and isolated rats. This has been reported by Clarke & R.Kumar (1983) that initially nicotine(0.1,0.2,0.4mg/kg s.c.) produces depression of locomotor activity and ataxia in the first 20min but increased activity later in session due to the tolerance to nicotine\textsuperscript{115}. Even nicotine (0.1-1 mg/kg) administered s.c. to rats acutely or repeatedly (eight injections at 48-h intervals) produced transient hypoactivity, followed by dose-related hyperactivity\textsuperscript{116}.

Nicotine given by subcutaneous route showed delayed action on locomotion at 20min in normal as well as in isolated rats. Nicotine by inhalation on the other hand shows faster onset
of action at 10 mins. This can be explained by the fact that nicotine by inhalation, undergoes faster absorption and higher concentrations in brain are reached earlier as compared to nicotine(s.c.). Nicotine is absorbed readily through the skin, mucous membranes and lungs. The pulmonary route of administration produces significant CNS effects in just 7 seconds. P.Popik (2005) suggested that early decrease in locomotor activity response induced by nicotine could be due to its adaptive action on dopaminergic system while imipramine requiring longer time to develop, may be due to the adaptation of noradrenergic system. As a consequence, imipramine showed delay in action on locomotor activity\(^2^2\).

Thus findings of our study on locomotor activity in normal rats are in agreement with previous studies\(^2^2,1^1^5,1^1^6\).

In isolation induced hyperactivity model of depression, imipramine and nicotine reduced locomotor activity. Thus antidepressant activity of standard antidepressant drug imipramine and nicotine in isolated rats was observed in this model.

In our study, it is interesting to note that imipramine showed antidepressant effect after 7 days of treatment in both models of depression. However, these results are contradictory to those reported by P.Popik (2005), where antidepressant effect of imipramine was observed after 2 weeks treatment with imipramine alone\(^2^2\).

However it may be noted that in present study, single dose of nicotine by inhalational route showed comparable antidepressant effect to that of 7 days treatment of imipramine.

In this study, it was interesting to note that, single dose of nicotine given by inhalation showed antidepressant activity comparable to that of seven days treatment with imipramine even in both learned helplessness model as well as isolation induced hyperactivity model.

Combination of imipramine and inhalational nicotine in single dose or 7 days treatment showed additive, if not potentiating effect of imipramine in isolation induced hyperactivity model. This additive antidepressant effect of inhalational nicotine was observed only with 7 days treatment in learned helpless model.

In the present study, single dose of nicotine by subcutaneous route in both models of depression i.e. isolation induced hyperactivity and learned helplessness model showed antidepressant activity. Of interest, the magnitude of this response was equivalent to that observed with the standard antidepressant drug, imipramine.
When nicotine single dose was given by s.c. route with imipramine, no additive effect was seen during the observed period. It is difficult to explain this since both imipramine for 7 days and single dose of nicotine (s.c.) have independently demonstrated antidepressant action.

The main finding of the present study was that, single dose of inhalational administration of nicotine showed comparable results to that of seven days treatment with standard antidepressant drug imipramine. This has clinical significance since at present no satisfactory drug treatment is available for an acute episode of depression.

Previous studies of the time-course of the effects of imipramine have reported that antidepressant action of imipramine after 2 weeks treatment with imipramine and after 1 week of treatment with a combination of nicotine and imipramine. In a previous study, potentiation of apomorphine induced hyperactivity was used to study characteristic effect of antidepressants as described by Spyraki 1981. Antidepressant action of imipramine or nicotine after one week can be explained by use of different model used for evaluation of antidepressant action.

Ferguson et al (2000), reported that nicotine did not influence the learned helplessness response, though a subtype-selective nicotinic acetylcholine receptor agonist produced antidepressant like effect in this model. In other studies on learned helplessness model with subcutaneous implantation of nicotine(1.5mg/kg/day), reduced number of escape failure on day 14. This indicates a delayed antidepressant effect of nicotine in this model. However, they failed to observe any effect after 7 days treatment with nicotine(s.c.) implant. It may be noted that in our study even single dose of subcutaneous nicotine demonstrated antidepressant effect. This discrepancy may be due to subcutaneous implant used in their study and slow release of nicotine from the implant.

Effects of these treatments were studied on three behavioral parameters i.e. persistent sniffing, paw licking, intense biting. Effects of nicotine by both routes on behavioral parameters were similar to imipramine. Bhatawadekar AD et al, have reported that acute administration of nicotine(0.4mg/kg s.c.) potentiated amphetamine induced stereotyped behaviour. It is well established that in rats stereotyped sniffing, licking and biting behaviour are probably due to the stimulation of dopaminergic mechanisms in the striatum.

In the present study nicotine by both routes demonstrated antidepressant activity in the models of depression used. The mechanism of antidepressant action of nicotine has not been reported.
in the literature. There are controversial reports regarding action of nicotine in haloperidol induced catalepsy and lithium induced head twitches model 11,117. It was therefore planned to study the effect of nicotine on brain monoamines i.e. DA,5-HT and NA. The results from the present study suggest that nicotine modulates the behaviour mediated via monoamine neurotransmitters like DA,5-HT, and NA.

Haloperidol causes extrapyramidal symptoms by inhibiting D2 receptors and the drugs increasing release of DA inhibit development of these symptoms88,118. As reported by Saher (2007) nicotine(1.2mg/kg s.c.) administered 15min before test, produced the reversal of anhedonia in mice exposed to 3-weeks application of stressors119. However, model of anhedonia produced an increase in the expression of pre and post-synaptic dopamine D2 receptors in the nucleus accumbens120. In the present study,haloperidol induced catalepsy was inhibited with subcutaneous nicotine, but this effect diminished with time when nicotine was administered by subcutaneous route. However, nicotine administered inhalationaly reduced catalepsy at all time points indicating that nicotine administered inhalationaly increased levels of dopamine for a longer time in rat brain. This effect is predominantly seen with inhalational nicotine as compared to subcutaneous nicotine.

According to previous reports, standard antidepressant drug imipramine, increases dopamine, serotonin and noradrenergic activity51,63,87,91.121.

Ghulam Moinuddin (2012) and Abdel-Salam, O. M. et al. (2007) have reported anticataleptic effect of imipramine in haloperidol induced catalepsy model88,122. In the present study nicotine administered by both routes demonstrated anticataleptic effect. Our result are similar to these findings as reported with imipramine.

The behavioral model used in our studies to evaluate serotonergic activity of nicotine was lithium induced head twitches. Lithium produces head twitches by increasing the synaptic concentration of serotonin90. Head twitches are used to study 5-HT neuron activity, several drugs induce these in rats i.e. 5-hydroxytryptophan(5-HTP) by increasing the free concentration of 5-HT at its receptor site90. In the present study, we have reported that head twitches were seen in rats treated with lithium chloride. Nicotine administered by both routes potentiated the head twitches in rats. This indicates increase in synaptic serotonergic activity with both subcutaneous and inhalational administration of nicotine.
It has been amply reported that antiadrenergic drugs like clonidine, α-methyldopa, and reserpine have depression as their side effect\textsuperscript{40,62}. Clonidine induced sedation is a behaviour mediated via noradrenaline\textsuperscript{91}. The sedative action of clonidine is due to decreased release of NE due to synaptic stimulation of presynaptic inhibitory α2 adrenoceptors. Antidepressants could counteract this effect by inhibiting reuptake of NE and by blocking (e.g. mianserine) the presynaptic α2 adrenoceptors\textsuperscript{91}. Specific α2-adrenoceptor agonist clonidine increased sleeping time in rats. The same effect was seen with the β-antagonist propranolol which impaired righting reflex in rats\textsuperscript{56,93}.

Numerous studies have suggested that propranolol administration results in progressive decline in the levels of catecholamines in different parts of the brain. Similarly, dopamine β-hydroxylase activity was also decreased in these parts of the brain\textsuperscript{123}.

In the present study, nicotine administration reversed the sedation induced by clonidine. This reversal of sedative action of clonidine by nicotine may be because of its CNS stimulant action. However, sedation or impaired righting reflex produced by blockade of β receptor with propranolol is not reversed by single dose of nicotine.

These results indicate that reversal of sedative action of clonidine after nicotine is mediated through α2 receptors but not through β receptors directly. Popik et al(2005) have reported downregulation of β2 receptors after 7 days treatment with nicotine. In our study, single dose of nicotine was administered inhalational route. Single dose is unlikely to downregulate the receptors and this explains the failure of action of nicotine on β receptors\textsuperscript{22}.

Studies in the pharmacodynamic models therefore indicate an increase in DA,5-HT, and NA activity. This is proved mechanism of antidepressant action of TCAs and SSRI.

The principal findings of our study therefore confirm antidepressant action of nicotine reported in previous studies. Increase in DA,5-HT, and NA activity in pharmacodynamic models is observed. Therefore, levels of neurotransmitters in rat brain tissue were estimated.

Before estimation of neurotransmitters in rat brain tissue, rats were isolated for 15 days. After isolation, as expected levels of neurotransmitters i.e. DA,NA,5-HT and GABA were significantly reduced compared to normal rats. After isolation i.e. in depressed rats, levels of neurotransmitters in vehicle treated group were compared with study treatment.

To summarize, levels of DA, NA, serotonin (5-HT), GABA were significantly decreased in brain tissue of isolated (depressed) rats. Increase levels of these neurotransmitters were seen in
groups treated with standard antidepressant drug imipramine and nicotine administered by inhalational and subcutaneous route. Effect of single dose of subcutaneous nicotine on neurotransmitters levels is of lesser magnitude. Increased levels of DA, 5-HT, NA substantiate the findings of our study confirming the effect of imipramine and nicotine on these neurotransmitters observed in pharmacodynamic models used.

Both the study treatments increased these monoamines as well as GABA in isolated rats which were depressed. However, as compared to imipramine, magnitude of these effects differed on different neurotransmitters levels. Increase in DA and 5-HT is seen with nicotine (inhal) but the magnitude is less as compared to imipramine, though not statistically significant. Here nicotine was administered as a single dose. Reports from literature indicate increase in DA levels after chronic treatment with nicotine. This may be responsible for reinforcement action and nicotine in addiction.

It has been reported that dopaminergic depletion is involved at least in major depression. Dmasma et al. (1989) reported that chronic treatment with nicotine produced adaptation of the dopaminergic system by increasing the extracellular concentrations of dopamine in mesolimbic areas.

In brain region, the nicotinic receptors can regulate the release of dopamine via the dopamine transporter. Thus nicotine stimulates the release of dopamine in pleasure circuit and increases extracellular levels of dopamine in nucleus accumbens. This has been reported with chronic administration of nicotine.

Noradrenaline levels decreased in brain tissue of isolated rats i.e. in depressed rats. Single dose of nicotine by both route, on the other hand has increased NA and GABA levels to the same extent as imipramine. These neurotransmitters may therefore play a crucial role in antidepressant action of nicotine. In our study, it was observed that antidepressant action of nicotine by increasing noradrenergic activity may be mediated by blockade of central α2 receptor.

GABA(γ-amino butyric acid) levels reduced in isolated rat brain tissue i.e. in depressed rats. Single dose of nicotine by inhalation route has increased GABA levels to the same magnitude as imipramine.

Thus, imipramine and nicotine by inhalational route may reduce symptoms of depression by increasing NA levels in depressed rats. At the same time, nicotine (s.c.) increased NA levels,
though to a lesser extent.

There are reports available on the preclinical and clinical studies involving GABA neurotransmission in mood disorders. GABA may activate the dopaminergic system, depending upon the brain region and the duration of GABA stimulation. A complex interaction between GABA and noradrenergic transmission has been reported in previous animal studies. It has been reported that serotonin release is increased by stimulation of GABA receptors in rat suprachiasmatic areas. Preclinical studies have suggest that GABA levels may be decreased in animal model of depression and low plasma and CSF GABA levels in depressed patients have been reported.

Nicotine also alters the functions of some of the neurotransmitters implicated in the pathogenesis of some of the major psychiatric disorders. These include dopamine, noradrenaline, serotonin (5-HT), GABA and endogenous opioid peptides. These effects could be presynaptic, preterminal or on cell body nicotine receptors, rather than being mediated through neurotransmission wherein pre-synaptically released acetylcholine acts on postsynaptic, junctional nAChRs to cause neuronal firing.

Future studies will explore the relationship between monoaminergic, serotonergic, GABAergic and cholinergic through central nAchRs system and should further clarify the potential mechanisms of nicotine involved in the neurotransmission in mood disorders.

To conclude therefore, our study demonstrates the antidepressant effect of nicotine by both subcutaneous and inhalational route. However, inhalational nicotine produced early and significant effect even with single dose. This action of nicotine may be attributed to increasing brain NA levels which is mediated by inhibiting central α2 receptors and increasing GABA levels.

Today there is no treatment except ECT (Electroconvulsive therapy) for acute episode of depression with suicidal tendency. The available standard antidepressant drugs i.e. TCA and SSRI have delayed onset of action. So inhalational nicotine or nicotine analogues may have clinical advantage in such situation. Partial nicotine receptor (nAChRs) agonists varenicline, sazetidine and cytisine are potent partial agonists at α4β2 subtype but they have very low affinity for other nAChRs subunits like α7. They are used in the management of depression and they have delayed onset of action as that of standard antidepressant like TCA and SSRI and require longer duration for therapeutic benefit. In our study, it was observed that single
dose of nicotine by inhalation showed faster onset of action in animal models of depression. Therefore, it is suggested that single dose of inhalational nicotinic analogues may be the drugs of future in the management of acute episode of depression with suicidal tendency.