TO EVALUATE THE ROLE OF NICOTINE IN DEPRESSION
BY USING ANIMAL MODELS.

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ABSTRACT
To study antidepressant action of nicotine in animal model of depression. The animal model for depression, used was ‘Isolation induced hyperactivity in rats’. Doses given were vehicle 1ml/kg (intra-peritoneal), imipramine10mg/kg (intra-peritoneal), nicotine0.4mg/kg (subcutaneous), nicotine 0.2mg/kg (inhalational) Nicotine administered by subcutaneous route showed significant reduction in hyperactivity at 10 and 20 minutes when compared with that of vehicle (control) group. When it was compared with imipramine, it showed significant reduction in hyperactivity at 10 minutes. Nicotine administered by inhalation route showed significant reduction in hyperactivity at 10 min and at 30, 40 50 minutes when compared with that of control group. When compared with imipramine, it showed significant reduction in hyperactivity at 10 minutes and it showed comparable effect with that of imipramine at 30, 40, and 50 minutes. Nicotine administered by inhalation route produced significant reduction in hyperactivity at 10, 20, 30, 40,50minutes, when compared with that of nicotine administered by subcutaneous route. Combination with imipramine acute or chronic administration of nicotine by inhalational route showed significant reduction in hyperactivity, when compared with imipramine treated rats. Imipramine treated rats showed significant changes in behavior with persistent sniffing, intense biting and paw licking when it compared with vehicle treated rats. Behavioral changes in nicotine treated rats showed significant change sat persistent sniffing, intense biting, and paw licking. Effects of nicotine with imipramine were studied on all the above parameters. Nicotine administered by subcutaneous and inhalation route showed significant antidepressant activity.

Key words Behavioral Changes, Depression, Isolation-Induced Hyperactivity, Nicotine

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INTRODUCTION:
Nicotine derived from the leaves of tobacco belonging to the family *Nicotianatabacum* and has been in use for centuries. Nicotine acts on nicotinic receptors in the autonomic ganglia, adrenal medulla and neuromuscular junction. The specific sites for binding in the brain are the hypothalamus, hippocampus, thalamus, midbrain, brainstem and cerebral cortex. Nicotine also binds to receptors in the nigrostriatal and mesolimbic dopaminergic neurons. Nicotine receptors when stimulated release acetylcholine, noradrenaline, dopamine, serotonin, vasopressin, growth hormone and ACTH. In lower concentrations, nicotine is a stimulant. This is one of the main factors leading to the pleasure and habit forming qualities of tobacco smoking\(^1\).

According to the SalinPascual et al in 2002, nicotine patches can improve the mood in depressed patients \(^2\). According to Bonnie Spring et al (2008), depressed people are more likely to smoke and are more resistant to quit smoking \(^3\).

However, it is unclear, if nicotine or other chemicals directly affect the brain of a depressed person. Nicotine may have antidepressant properties and smokers self-medicate underlying depression \(^4, 5\). Epidemiological findings suggest that smokers more often demonstrate depressive symptoms than non-smokers and depressed patients are less likely to cease smoking \(^6\). As reported by Ferguson et al (2000) nicotine appeared not to influence the learned helplessness response, though a subtype selective nicotinic acetylcholine receptor agonist produced antidepressant like effect. Preclinical and clinical data regarding antidepressant action of nicotine are ambiguous \(^7\).

MATERIALS AND METHODS
Experimental protocol was approved by our Institutional Animal Ethical Committee (IAEC). Wistar rats weighing 200-250gm housed in polypropylene cages (single rat/cage) were used. They were fed pellet diet and water *ad-libitum*. The rats were maintained under standard conditions of temperature (25\(^0\)C ±5\(^0\)C) and relative humidity (55±10%). Rats of either sex were used. Rats were divided into eight groups. 10 rats in each group. (Table 1)

**Evaluation of antidepressant activity in rats**
Antidepressant action of nicotine was studied in isolation induced hyperactivity model in rats by using photoactometer \(^8\).

**Drugs**
Imipramine HCl (Sun Pharmaceutical Industries Ltd, Mumbai), Nicotine Hydrogen Tartrate (Sigma-Aldrich, Poland) were dissolved in distilled water (vehicle). Doses given were vehicle
1ml/kg (intraperitoneal) imipramine 10mg/kg (intraperitoneal), nicotine 0.4mg/kg (subcutaneous), nicotine 0.2mg/kg (inhalational).

### Table 1: Study treatment design

<table>
<thead>
<tr>
<th>Study Treatment In Each Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1              Vehicle control</td>
</tr>
<tr>
<td>Group 2              Imipramine 10mg/kg i.p. for 7 consecutive days</td>
</tr>
<tr>
<td>Group 3              Nicotine 0.4mg/kg s.c.</td>
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<tr>
<td>Group 4              Nicotine 0.2mg/kg inhalational</td>
</tr>
<tr>
<td>Group 5              Imipramine + Acute Nicotine (s.c.) Combination</td>
</tr>
<tr>
<td>Group 6              Imipramine + Chronic Nicotine (s.c.) Combination</td>
</tr>
<tr>
<td>Group 7              Imipramine + Acute Nicotine (inhalational) Combination</td>
</tr>
<tr>
<td>Group 8              Imipramine + Chronic Nicotine (Inhalational) Combination</td>
</tr>
</tbody>
</table>

### Design of experiments

In this model of depression, adult Wistar rats were socially deprived for a period of 15 days. Rats were housed singly in cages (38cm × 26cm × 20cm) without any visual or auditory contact with their normally housed counterparts for 15 days. The locomotor activity score was tested after 15 days of isolation by keeping the rat in a photoactometer. The locomotor activity was recorded on digital recorder as rat moved and crossed beam. Reading was noted for 1 minute every 10 minutes up to 50 minutes.

After isolation, hyperactivity was compared with that of vehicle treatment and imipramine treated rats. In acute study, single dose of nicotine (subcutaneous) or nicotine (inhalational) were administered at the end of 7-days of administration of imipramine and effect on locomotor activity and behavioral changes was observed. In chronic study, imipramine was administered for 7 consecutive days with nicotine (subcutaneous) or nicotine (inhalational) and effects on locomotor activity and behavioral changes were observed at the end of treatment.

The locomotor activity score was tested after 15 days of isolation, with vehicle, imipramine, nicotine (subcutaneous) and nicotine (inhalation) using actophotometer. Effect of combination of acute and chronic administration of nicotine with imipramine was studied on locomotor activity after isolation. Simultaneously behavior parameters i.e. sleep reduced response to external stimuli; ambulatory behavior, stereotypy and posture were studied in all the study groups.

### Data presentation and statistics

Locomotor activity score/min every 10 minutes up to 50 minutes was measured. The results were expressed as mean±sd. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey test, using Primer of Biostatistics’< 0.05 was considered to be statistically significant. Behavioral parameters were analyzed by Mc-Nemars test for
paired data and Fishers exact test for unpaired data, using Primer of Biostatistics $< 0.05$ was considered statistically significant $^{10}$.

RESULT AND DISCUSSION

In this model, vehicle (control) has shown isolation induced hyperactivity till 50min when compared with both imipramine as well as nicotine treated groups. In imipramine treated group, isolation induced hyperactivity was reduced till 50min. When compared with vehicle control, acute administration of nicotine (s.c.) showed significantly reduced hyperactivity at 10 minutes $72.9 \pm 3.98$ (vehicle control) to $56.7 \pm 5.77$ per min and $75.8 \pm 5.03$ (vehicle control) to $60.7 \pm 7.23$ per min at 20 minutes (Table 2, Figure 1). After a sudden increase in activity at 30 min, there was a gradual decrease to $55.2 \pm 3.55$ at 50min. When compared with vehicle control, acute administration of nicotine (inhalational route) showed significantly reduced hyperactivity at 10 minutes $72.9 \pm 3.98$ (vehicle control) to $30.2 \pm 3.68$ per min. In vehicle control group, locomotor activity was at $70.2 \pm 2.53$ (vehicle control) per min at 30 min, $62.8 \pm 5.18$ per min at 40 min, $59.5 \pm 6.74$ per min at 50 min. In nicotine (inhalational) group the locomotor activity was $56.7 \pm 2.63$ per min, $54.1 \pm 2.42$ per min, $48 \pm 2.26$ per min at 30, 40 and 50 min respectively. At 30 & 40 min, the results were comparable with imipramine. However, at 50 min, when compared with imipramine, nicotine administered by inhalation route decreased hyperactivity significantly (Table 2, Figure 1).

![Figure 1: Locomotor activity (per minute) in rats after treatment.](image-url)
Table 2: Result of nicotine treatment

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Vehicle (sc) (Mean ± sd)</th>
<th>Imipramine (i.p.) (Mean ± sd)</th>
<th>Nicotine (sc) (Mean ± sd)</th>
<th>Nicotine (inhalation) (Mean ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>69.9± 2.56</td>
<td>68.5 ±2.17</td>
<td>68.7±3.47</td>
<td>66 ± 7.79</td>
</tr>
<tr>
<td>10</td>
<td>72.9± 3.98</td>
<td>68.2 ± 2.10 ●</td>
<td>56.7±5.77▲</td>
<td>30.2 ± 3.68 ■</td>
</tr>
<tr>
<td>20</td>
<td>75.8±5.03</td>
<td>58.8±4.64 ●</td>
<td>60.7±7.23 #</td>
<td>70.4 ± 2.32 *</td>
</tr>
<tr>
<td>30</td>
<td>70.2±2.53</td>
<td>57.6±4.12 ●</td>
<td>69.8±5.12</td>
<td>56.7 ± 2.63 *</td>
</tr>
<tr>
<td>40</td>
<td>62.8±5.18</td>
<td>55.6±4.58 ●</td>
<td>61.7±5.38</td>
<td>54.1 ± 2.42 *</td>
</tr>
<tr>
<td>50</td>
<td>59.5±6.74</td>
<td>52.3±4.57 ●</td>
<td>55.2±3.55</td>
<td>48 ± 2.26 *</td>
</tr>
</tbody>
</table>

● = Vehicle Vs Imipramine (i.p. - intraperitoneal) (P < 0.05)
# = Vehicle Vs Nicotine (sc - subcutaneous)
▲ = Vehicle Vs Nicotine (inhalational)

When compared with imipramine, the difference was not significant with acute or chronic administration of nicotine with imipramine combination administered by subcutaneous route.

When compared with imipramine, acute administration of nicotine (inhalational route) with imipramine showed significantly reduced hyperactivity at 10 minutes 68.2 ± 2.10(imipramine alone) to 60.1±4.07 per min at 30 min; 57.6±4.12(imipramine alone) to 52±3.23 per min at 30 min; 55.6±4.58 to 50.3±2.79 per min at 40 min; 52.3±4.57 to 43.5±3.27 per min at 50 min. When compared with imipramine, chronic administration of nicotine (inhalational route) with imipramine, showed significantly reduced hyperactivity at 10 minutes 68.2 ± 2.10(imipramine alone) to 52.3±4.83 per min; 58.8±4.64(imipramine alone) to 48.6±3.81 per min at 20 min; 57.6±4.12(imipramine alone) to 46.4±2.63 per min at 30 min; 55.6±4.58(imipramine alone) to 41.8±1.81 per min at 40 min; 52.3±4.57(imipramine alone) to 39.2±2.44 per min at 50 min (Table 2, Figure 2).

Table 3: Results of combination of imipramine + nicotine treatment

<table>
<thead>
<tr>
<th>Isolation induced hyperactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (minutes)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>20</td>
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<tr>
<td>30</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>50</td>
</tr>
</tbody>
</table>

● = Imipramine (i.p.) Vs imipramine +Acute Nicotine(sc - subcutaneous) (P < 0.05)
▲ = Imipramine VsImipramine +Acute Nicotine (inhalation)
# = Imipramine VsImipramine + Chronic Nicotine(inhalation)
Figure 2: Locomotor activity (per minute) in rats after combination with imipramine + nicotine treatment.

In this study, there were some behavioral changes seen. Imipramine treated rats showed significant changes in behavior with persistent sniffing, intense biting and paw licking when it compared with vehicle treated rats. Behavioral changes in nicotine treated rats showed significant changes at persistent sniffing, intense biting, and paw licking. (Table 4 and 5)

Table 4: Results of changes in behavioral pattern after drug treatment in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Persistent sniffing</th>
<th>Paw licking</th>
<th>Intense biting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>07</td>
<td>07</td>
</tr>
<tr>
<td>Imipramine</td>
<td>01</td>
<td>01</td>
<td>00</td>
</tr>
<tr>
<td>Nicotine (sc)</td>
<td>02</td>
<td>03</td>
<td>01</td>
</tr>
<tr>
<td>Nicotine (inhalational)</td>
<td>00</td>
<td>02</td>
<td>01</td>
</tr>
</tbody>
</table>

Table 5: Results of changes in behavioral pattern after combination treatment in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Persistent sniffing</th>
<th>Paw licking</th>
<th>Intense biting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine</td>
<td>01</td>
<td>01</td>
<td>00</td>
</tr>
<tr>
<td>Imipramine + Acute Nicotine(sc)</td>
<td>02</td>
<td>00</td>
<td>03</td>
</tr>
<tr>
<td>Imipramine + Chronic Nicotine(sc)</td>
<td>01</td>
<td>02</td>
<td>00</td>
</tr>
<tr>
<td>Imipramine + Acute Nicotine(inhalation)</td>
<td>00</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Imipramine + Chronic Nicotine(inhalation)</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
</tbody>
</table>

In the present study, imipramine has demonstrated antidepressant activity in isolation induced hyperactivity model as observed by decrease in reduction in isolation-induced hyperactivity starting at 10 minutes and lasting for 50 minutes when compared with vehicle treated group.
As compared to vehicle treated group, nicotine administered by subcutaneous route showed antidepressant activity but this action was short lasting, upto 20 minutes. However, nicotine administered by inhalational route has shown antidepressant activity comparable to imipramine, except at 20 minutes. At all other time points, nicotine has shown antidepressant activity till 50 min and is comparable to imipramine. But at 20 min nicotine administered by inhalational route showed a sudden increase in activity.

Antidepressant action of imipramine is seen only after chronic treatment, while acute administration of nicotine showed antidepressant activity. This is due to increase dopamine activity, while imipramine acts by increasing catecholamines levels. Antidepressant activity of nicotine administered by inhalational route is comparable to imipramine at 30 to 50 minutes. Nicotine given by inhalational route has shown long lasting antidepressant action compared to nicotine administered by subcutaneous route.

Changes in locomotor activity that occur after the administration of nicotine and other nicotinic receptor agonists in rats have been extensively documented. An increase in locomotor activity occurs consistently after nicotinic agonist administration followed by a subsequent challenge, a phenomenon known as behavioral sensitization. The increase in locomotor activity during sensitization is recognized as a plastic event related to the modulation of dopamine release mainly in the mesolimbic pathway by β2 subunit-containing nicotinic receptors.

Nicotine-induced increase in locomotor activity and changes in behavioral pattern depend on an intact dopaminergic system. Nicotinic AChRs containing α6 and β2 subunits are highly expressed in VTA dopamine neurons, and seem to be involved in both nicotine-induced locomotor activation.

Nicotine (inhalational) with imipramine decreased isolation induced hyperactivity, which is probably indicative of additive antidepressant action with imipramine due to nicotinic action with α4β2 receptors.

CONCLUSION

This study has demonstrated significant antidepressant action of nicotine, when administered by inhalational route. Involvement of dopaminergic system is also likely as reported by Piotr Popik et al (2004) Involvement of nicotinic α4β2 receptor subunit in antidepressant action of nicotine is possible and this may be responsible for smoking cessation with nicotinic α4β2 agonist. This possibility opens up new avenues for treatment of smoking cessation and depression.
REFERENCES


Effect of Nicotine on Brain Gaba levels in Depressed rats.

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1. Department of Pharmacology, Pd. Dr. D.Y. Patil medical college, Dr. D.Y. Patil University, Pimpri, Pune-18
2. Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune-38

ABSTRACT

Effect of nicotine on brain GABA levels in depressed rats. The present study was planned: to study effect of nicotine on brain GABA levels in depressed rats. to compare the effect of nicotine and imipramine on brain GABA levels. Isolation induced hyperactivity model was used to induce depression in rats. Five groups of 10 rats each were taken. Vehicle (D/W) treated rats before and after isolation were considered as baseline reading. Compared results of depression induced animal with results of animal without depression. Following drug treatments were administered: Rats from natural habitat was considered as before isolation. This group was used for normal GABA levels in rat brain. Vehicle (D/W) (1ml/kg) and imipramine (10mg/kg) were administered intraperitoneally. Nicotine was administered in a dose of 0.4mg/kg and 0.2mg kg by subcutaneous or inhalational route respectively. Brain GABA levels were estimated by fluorimetric method. In this model of depression, vehicle treated rats after isolation significantly reduced brain GABA levels as compared to vehicle before isolation. Results of imipramine treated rats after isolation showing significantly increased in brain GABA levels as compared to vehicle treated rats after isolation. Nicotine administered by inhalational route showed increase in brain GABA levels as compared to vehicle treated rats after isolation. Nicotine administered subcutaneously increased brain GABA levels as compared to vehicle treated rats after isolation. Imipramine and nicotine (inhalation) showed comparable results with normal GABA level i.e. before isolation rats. GABA level reduced in depressed rats. Imipramine, nicotine(inhalation) and nicotine(sc) increased brain GABA level in depressed rats.

Keywords: Depression, Isolation induced hyperactivity, Brain GABA level, Nicotine

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INTRODUCTION

The potential role of GABAergic dysfunction in mood disorders was first proposed by Emrich et al based on the efficacy of valproate in the treatment of bipolar patients. GABA is an inhibitory neurotransmitter present almost exclusively in the central nervous system (CNS), distributed across almost all brain regions, and expressed in interneurons modulating local circuits. GABA transmission is present in interneurons modulating local neuronal circuitry, including noradrenergic, dopaminergic, and serotonergic neurons. After Emrich’s hypothesis, several animal and human studies have evaluated the potential role of GABAergic abnormalities in the pathophysiology of mood disorders. Preclinical studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in depressed patients.

GABAergic modulation of neuronal activity

GABA may activate the dopaminergic system, depending upon the brain region and the duration of GABA stimulation. It has been reported that muscimol, which is a GABA agonist, may reduce the immobility time in the behavioral despair model for depression by activating the rat dopaminergic system.

Animal studies have reported a complex interaction between GABAergic and noradrenergic transmissions. It has been reported that GABA, progabide, and fengabine induce norepinephrine neuronal activity in rat brains. GABA_A and GABA_B receptor activation may increase and decrease norepinephrine release in rat cortex and hippocampus respectively.

GABA-serotonin relationship may be more complex. It has been reported that serotonin release is increased by stimulation of GABA receptors in rat suprachiasmatic areas.

MATERIAL AND METHODS

Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). Wistar rats weighing 200-250gm housed in polypropylene cages (single rat/cage) were used. They were fed pellet diet and water ad-libitum. The rats were maintained under standard conditions of temperature (25±0°C) and relative humidity (55±10%). Rats of either sex were used. Rats were divided into 10 groups. 10 rats in each group.

Evaluation of antidepressant activity in rats

Antidepressant action of nicotine was studied in ‘isolation induced hyperactivity model’ in rats.

Drugs

Imipramine HCl (Sun Pharmaceutical Industries Ltd, Mumbai), Nicotine Hydrogen Tartrate
(Sigma-Aldrich, Poland) were dissolved in distilled water (vehicle). The study treatment were administered as follows: vehicle 1ml/kg (intraperitoneal) imipramine 10mg/kg (intraperitoneal), nicotine 0.4mg/kg (subcutaneous), nicotine 0.2mg/kg (inhalational). As shown in Table -1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle control (before isolation )</td>
</tr>
<tr>
<td>Group 2</td>
<td>Vehicle control (after isolation )</td>
</tr>
<tr>
<td>Group 3</td>
<td>Imipramine (10mg/kg i.p.) for 7 consecutive days (after isolation)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Nicotine (0.4mg/kg s.c.) single dose (after isolation)</td>
</tr>
<tr>
<td>Group 5</td>
<td>Nicotine (0.2mg/kg inhalation) single dose (after isolation)</td>
</tr>
</tbody>
</table>

**Design of experiments**

In this model of depression, adult Wistar rats were socially deprived for a period of 15 days. Rats were housed singly in cages (38cm × 26cm × 20cm) without any visual or auditory contact with their normally housed counter parts for 15 days. After isolation, rats became hyperactive. This increase in locomotor activity was measured by using digital photoactometer.  

**Biochemical evaluation by estimation of brain GABA**

Depressed rats were sacrificed before and after completion of drug treatment period. Brain was isolated immediately and transferred to homogenization tube containing 5 ml of 0.01 M hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 ml of ice cold absolute alcohol and kept for 1 h at 0 °C. The content was centrifuged for 10 min at 16000 rpm, supernatant was collected in petridish. Precipitate was washed with 5 ml of 75% alcohol for three times and washes were combined with supernatant. Contents in petridish were evaporated to dryness at 70 °C on water bath under stream of air. To the dry mass 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0 ml) was separated and 10 µl of it was applied as spot on Whatman paper (No.41).

The mobile phase consisted of n-butanol (50 ml) acetic acid (12 ml) and water (60 ml). The chamber was saturated for half an hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 h at 90 °C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on water bath for 5 min. Water (5.0 ml) was added to solution and kept for 1h. Supernatant (2.0 ml) was decanted and absorbance was measured at 570 nm on fluorimetric detector.  

**Statistical Analysis**

In estimation of brain GABA level, data was analyzed by one-way analysis of variance.
(ANOVA) followed by Tukey test, using Primer of Biostatistics. P <0.05 was considered as significant.16

RESULTS AND DISCUSSION

Brain GABA level in rat before isolation was 53.28ng/g of brain tissue. Level of GABA was reduced to 21.47ng/g of brain tissue after isolation. Level of GABA in brain after isolation was significantly reduced as compared to before isolation( p < .001) shown in Table -2* Figure 2. GABA levels in the vehicle treated group after isolation was compared with the three study treatment group i.e. imipramine, nicotine(sc) and nicotine(inhalation) after isolation. GABA levels were significantly increased in imipramine treated groups to 48.18ng/g of brain tissue. This increase was highly significant as compared to the GABA levels in vehicle treated groups after isolation. Difference between results of vehicle and imipramine treated rats were highly significant( p < .001).This is depicted in Figure 1 and Figure 2.

(A)  (B)  (C)

Figure 1: – Comparison of effect

A. Vehicle control Vs Imipramine on GABA level in depressed rats.
B. Vehicle control Vs Nicotine (inhalation) on GABA level in depressed rats.
C. Vehicle control Vs Nicotine (sc) on GABA level in depressed rats.

Nicotine administered by subcutaneous and inhalational route increased in GABA level to 29ng/g and 42.92ng/g of brain tissue respectively. Nicotine administered by subcutaneous and inhalational route showed significantly increased brain GABA level as compared to vehicle treated rats after isolation i.e. in depressed rats. This is shown in Figure 1(B/C) and Table 2.
Figure 2: Brain GABA level in depressed rats after study treatment

### = Vehicle control (before isolation) Vs Vehicle control (after isolation) (p<0.001)

***= Vehicle control (after isolation) Vs imipramine (after isolation) (p<0.001)

*** = Vehicle control (after isolation) Vs Nicotine(inhalation) after isolation (p<0.001)

••• = Nicotine(sc) after isolation Vs Nicotine(inhalation) after isolation (p<0.001)

* = Vehicle control (after isolation) Vs Nicotine(sc) after isolation (p<0.05)

Table 2: Results of Brain GABA level after study treatment in depressed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Brain GABA level in depressed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle control (before isolation )</td>
<td>53.28±3.57</td>
</tr>
<tr>
<td>Group 2</td>
<td>Vehicle control (after isolation )</td>
<td>21.47±2.9###</td>
</tr>
<tr>
<td>Group 3</td>
<td>Imipramine (10mg/kg i.p.) for 7 consecutive days (after isolation)</td>
<td>48.18±3.17***</td>
</tr>
<tr>
<td>Group 4</td>
<td>Nicotine (0.4mg/kg s.c.) single dose (after isolation)</td>
<td>29±4.29*</td>
</tr>
<tr>
<td>Group 5</td>
<td>Nicotine (0.2mg/kg inhalation) single dose (after isolation)</td>
<td>42.92±5.04*** •••</td>
</tr>
</tbody>
</table>

### = Vehicle control (before isolation) Vs Vehicle control (after isolation) (p<0.001)

***= Vehicle control (after isolation) Vs imipramine (after isolation) (p<0.001)

*** = Vehicle control (after isolation) Vs Nicotine(inhalation) after isolation (p<0.001)

••• = Nicotine(sc) after isolation Vs Nicotine(inhalation) after isolation (p<0.001)

* = Vehicle control (after isolation) Vs Nicotine(sc) after isolation (p<0.05)

Imipramine and nicotine administered by inhalational route in depressed rats showed comparable results with GABA level in rats before isolation.

GABA level of nicotine treated by subcutaneous route was significantly less as compared to GABA level of imipramine treated rats as well as nicotine administered by inhalational route (p < 0.001).

The role of GABAergic dysfunction in mood disorders was first proposed 20 years ago. Preclinical studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in mood disorder patients. Also, antidepressants, mood stabilizers, electroconvulsive therapy, and GABA agonists...
have been shown to reverse the depression-like behavior in animal models and to be effective in unipolar and bipolar depression patients\(^2\). The hypothesis of reduced GABAergic activity in mood disorders may complement the monoaminergic and serotonergic theories, proposing that the balance between multiple neurotransmitter systems may be altered in these disorders\(^3\).

The previous study by the authors revealed the antidepressant effect of nicotine on isolation induced model of depression in rats. Rats with 15 days of isolation period demonstrated characteristic symptoms like increase in locomotor activity. The increase in locomotor activity was measured by using digital photoactometer in the previous study. Locomotor activity was significantly increased in control i.e. vehicle treated group after isolation. Locomotor activity was reduced with imipramine and nicotine treated rats in depression as compared to vehicle treated rats after isolation\(^17\).

Reduced GABA levels in rat nucleus accumbens, brain stem, and cortex have been reported after a session of forced swimming test. In learned helplessness model after suffering from an inescapable foot shock, animals are not able to perform simple escape tasks in a shuttle box, resembling the psychomotor impairment present in human depression. Sherman and Petty demonstrated that GABA injection into frontal neocortex and hippocampus reversed the learned helplessness reaction\(^2\).

In present study, brain GABA level of vehicle treated groups after isolation was reduced as compared to before isolation. Seven days treatment of imipramine and single dose of both nicotine administered by subcutaneous as well as inhalational route increased the brain GABA level in depressed rats. Effect on brain level of GABA in imipramine and nicotine administered by inhalational route was more pronounced as compared to nicotine administered by subcutaneous route. Hence, it can be concluded that nicotine elucidates its antidepressant effect in isolation-induced hyperactivity model of depression through modulation of brain GABA level in rats. This result is consistent with findings observed in depressed rats and the present results suggest that deficits in the brain GABA level are related to depression.

**CONCLUSION**

In conclusion present study suggest that the increased brain GABA level after nicotine in depressed rats may be useful to some extent at least. In addition, study on site-specific action of nicotine on brain GABA level needed.
REFERENCES


