AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

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

Rahul RaghunathBhalsinge*[1], Pratibha S. Worlikar[1], Abhijeet V. Tilak[1], Pavitra Raj Dewda[1]

1. Dept. of Pharmacology, D.Y.Patil Medical College,Hospital and Research Centre, Pimpri, Pune-411018.

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 antidepressantactivity.

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

*Corresponding Author Email: rahulbhalsinge@gmail.com

Received 6 December 2011, Accepted 19 December 2011

Please cite this article in press as: Bhalsinge R *et al.*, To Evaluate the Role of Nicotine in Depression by using Animal Models. American Journal of PharmTech Research 2012.

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 quits 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 (25^0C $\pm5^0$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) imipramine10mg/kg (intraperitoneal), nicotine 0.4mg/kg (subcutaneous),nicotine 0.2mg/kg (inhalational).

Table 1: Study treatment design

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

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. The locomotor activity score was tested after 15 days of isolation by keeping the rat in photoactometer. The locomotor activity was recorded on digital recorder as rat moved and crossed beam. Reading was noted for1minute 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 [8].

Data presentation and statistics

Locomotor activity score/min every 10 minutes upto 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 [9] 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± 3.98 (vehicle control) to 56.7±5.77 per min and 75.8±5.03 (vehicle control) to 60.7±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±3.55 at 50min.When compared with vehicle control, acute administration of nicotine (inhalational route) showed significantly reduced hyperactivity at 10 minutes 72.9± 3.98(vehicle control) to 30.2 ± 3.68 per min. In vehicle control group, locomotor activity was at 70.2±2.53 (vehicle control) per min at 30 min,62.8±5.18 per min at 40 min,59.5±6.74 per min at 50 min. In nicotine (inhalational) group the locomotor activity was 56.7 ± 2.63 per min, 54.1 ± 2.42 per min, 48 ± 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).

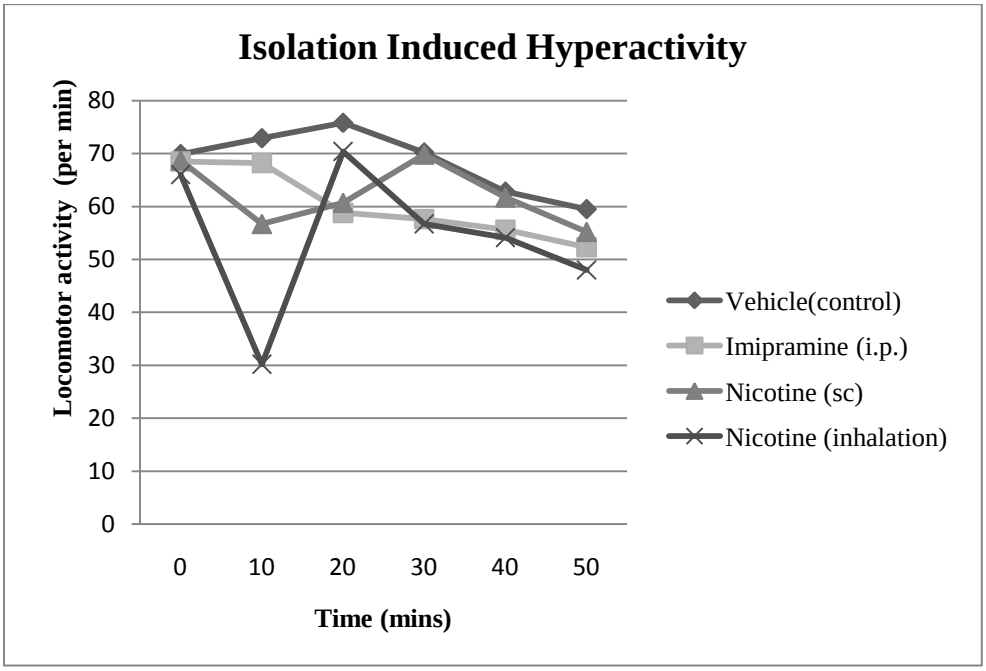

Figure1: Locomotor activity (per minute) in rats after treatment.

Table 2: Result of nicotine treatment

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

● = Vehicle Vs Imipramine (i.p. - intraperitoneal) (P < 0.05)
= Vehicle Vs Nicotine (sc - subcutaneous)
* = Vehicle Vs Nicotine (inhalational)
▲ = Imipramine Vs Nicotine (sc - subcutaneous)
♣ = Imipramine 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, 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±3.27per min at 50 min. When compared with imipramine, chronic administration of nicotine (inhalational route) with imipramine, showed significantly reduced hyperactivity at 10minutes 68.2 ± 2.10(imipramine alone)to 52.3±4.83 per min, 58.8±4.64(imipramine alone) to 48.6±3.81per 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 50min (Table 3,Figure 2).

Table 3: Results of combination of imipramine + nicotine treatment

Isolation induced hyperactivity					
Time (minutes)	**Imipramine (Mean ± sd)**	**Imipramine +Acute Nicotine(sc) (Mean ± sd)**	**Imipramine +Chronic Nicotine(sc) (Mean ± sd)**	**Imipramine +Acute Nicotine(inhalation) (Mean ± sd)**	**Imipramine +Chronic Nicotine(inhalation) (Mean ± sd)**
0	68.5 ±2.17	67.3±4.06	66.5±3.75	65±4.50	64.1±5.28
10	68.2 ± 2.10	66.4±3.44	66.1±3.35	60.1±4.07▲	52.3±4.83#
20	58.8±4.64	63.6±3.47●	61.3±3.33	55.4±3.81	48.6±3.81#
30	57.6±4.12	58.7±2.50	56.4±3.03	52±3.23▲	46.4±2.63#
40	55.6±4.58	54.9± 2.73	53.2±3.52	50.3±2.79▲	41.8±1.81#
50	52.3±4.57	51.8±2.39	50.9±3.18	43.5±3.27▲	39.2±2.44#

● = Imipramine (i.p.) Vsimipramine +Acute Nicotine(sc - subcutaneous) (P < 0.05)
▲ = Imipramine VsImipramine +Acute Nicotine (inhalation)
#= Imipramine VsImipramine + Chronic Nicotine(inhalation)

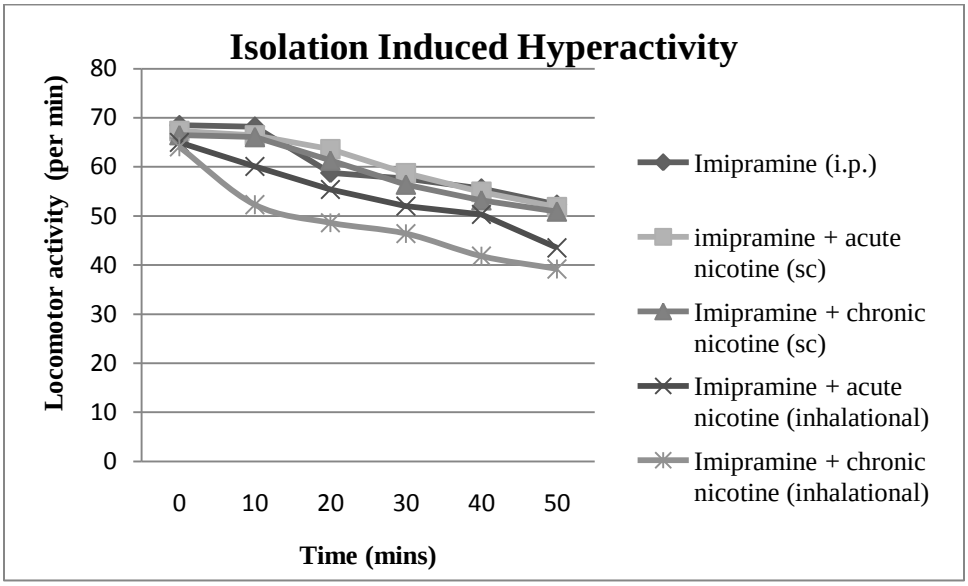

Figure 2:Locomotor activity (per minute) in rats after combination with imipramine + nicotine treatment.

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

No. of animals shows Changes in behavioral pattern after drug treatment			
Groups	**Persistent sniffing**	**Paw licking**	**Intense biting**
Vehicle	10	07	07
Imipramine	01	01	00
Nicotine (sc)	02	03	01
Nicotine (inhalational)	00	02	01

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

No. of animals shows Changes in behavioral pattern after combination treatment			
Groups	**Persistent sniffing**	**Paw licking**	**Intense biting**
Imipramine	01	01	00
Imipramine +Acute Nicotine(sc)	02	00	03
Imipramine +ChronicNicotine(sc)	01	02	00
Imipramine +Acute Nicotine(inhalation)	00	00	01
Imipramine +Chronic Nicotine(inhalation)	00	00	00

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 10minutes 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 20minutes. 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 50min 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[4].

Antidepressant activity of nicotine administered by inhalational route is comparable to imipramine at 30 to50minutes. 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 [11.] 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[12].

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[13].

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[14].

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 PiotrPopik et al(2004)[4] 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[14].This possibility opens up new avenues for treatment of smoking cessation and depression.

REFERENCES

1. Jain R, Mukherjee K. Biological basis of nicotine addiction. Indian J Pharmacol 2003; 35:281-9.
2. Rafael J, Pascual S. Nicotine antidepressant effects as a predictor of response to desipramine or fluoxetine in non-smoking major depressed patients. Salud Ment 2002; 25(02):16-20.
3. Spring B, Cook JW, Appelhans B. Nicotine effects on affective response in depression-prone smokers. Psychopharmacology.2008; 196(3): 461-71. doi: 10.1007/s00213-007-0977-7.
4. Popik P, Krawczyk M, Kos T, Nalep I. Nicotine produces antidepressant-like actions: Behavioral and neurochemical evidence. Eur J Pharmacol 2005; 51:128-133.
5. Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression.Neuroreport. 2002; 13(9):1097-106.
6. Turner JR, Castellano LM, Blendy JA. Nicotinic partial agonists varenicline and sazetidine-A have differential effects on affective behavior. J Pharmacol ExpTher 2010; 334(2):665-72.
7. Ferguson SM, Broadkin JD, Lloyd GK, Menzaghi F. Antidepressant-like effects of the subtype-selective nicotinic acetylcholine receptor agonist, SIB-1508Y, in the learned helplessness rat model of depression.Psychopharmacology (Berl.). 2000; 152(3):295-303.
8. Gupta SK. Drug screening methods of antidepressant. Drug screening methods; Jaypee Brothers, 2004: 77-83.
9. Rao KV. Biostatistics- A manual of statistical methods for use in health, nutrition and anthropology. Jaypee Brothers, 2nd Ed. 2007: 226-260.
10. Agresti A. An introduction to categorical data analysis. Wiley-Inter science; A John Wiley & Sons,Inc.,Pub. 2nd Ed. 2007: 244-275.
11. Abin-Carriquiry JA, Urbanavicius J, Scorza C. Increase in locomotor activity after acute administration of the nicotinic receptor agonist 3-bromocytisine in rats. Eur J Pharmacol 2010; 634:89–94.
12. Moretti M, Mugnaini M, Tessari M. Comparative study of the effects of the intravenous self-administration or subcutaneous mini pump infusion of nicotine on the expression of brain neuronal nicotinic receptor subtype. Mol Pharmacol. 2010; 78(2): 287-96.

13. Houezec JL. Nicotine: abused substance and therapeutic agent. J Psychiatry Neuroscience. 1998; 23(2):95-108.
14. Singh J, Budhiraja S. Partial nicotinic acetylcholine (α4β2) agonists as a promising new medications for smoking cessation. Indian J Pharmacol 2008;40(5):191-6