# EFFECT OF NICOTINE ON NOOTROPIC ACTIVITY AND OXIDATIVE STRESS

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#### **Summary**

The present work aims to investigate the effect of nicotine on learning and memory as well as on oxidative stress using both *invivo* and *in-vitro* methods. For the *in-vivo methods*, rats were appropriately grouped and tested for nootropic activity using various authenticated models and different doses in different routes. On the final day of study all the animals were sacrificed and the brain homogenates were estimated for antioxidant enzymes and lipid peroxidation levels. For the *in-vitro* studies, different concentrations of nicotine were used to measure hydroxyl radical scavenging and production as well as on Fe<sup>2+</sup> / Ascorbate and vitamin-E induced lipid peroxidation and protein denaturation. From the above study it was concluded that nicotine possessed nootropic activity as well as antioxidant activity to some extent.

Keywords: nootropic activity, nicotine, oxidative stress

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#### Introduction

Nicotine is an alkaloid present in the tobacco plant "*Nicotiana tobaccum*"<sup>(1,2)</sup>. Nicotine has always been regarded as medicinal and enjoyable at its usual low doses. Higher doses are toxic, even lethal - which is why nicotine is used around the world as an insecticide. Until recently, nicotine research has been driven primarily by nicotine's unparalleled power to keep people smoking, rather than its potential therapeutic uses.

However, nicotine-like other drugs, has a spectrum of effects, in addition to its addiction liability. Nicotine is similar to morphine, in that it has effects that may be therapeutically useful.

There is substantial evidence that oxidative stress is a causative at least ancillary factor in the pathogenesis of major or disease $^{(3)}$ . neurodegenerative diseases including Parkinson's Alzheimer's disease<sup>(4)</sup>, stroke, trauma and seizure. Nicotine has been reported to be therapeutic in some patients with certain neurodegenerative diseases and to have neuroprotective effects in the central nervous system. However, nicotine administration may result in oxidative stress by inducing the generation of reactive oxygen species in the periphery and central nervous system <sup>(5)</sup>. There is also evidence suggesting that nicotine may have antioxidant properties in the central nervous system<sup>(5)</sup>. The possibility that nicotine might be used to treat some symptoms of certain neurodegenerative diseases underlies the necessity to determine whether nicotine has pro-oxidant, antioxidant or properties of both.

Very few studies have examined the effects of nicotine on the CNS in relation to oxidative stress. Hence in our present investigation we aim to study the effect of nicotine on oxidative stress in the central nervous system by means of both *in-vivo* and *in-vitro* methods.

#### Methods

# **Drugs and chemicals**

Ascorbic acid, Potassium dihydrogen phosphate were obtained from Sisco Research Laboratories Pvt. Limited, India. Ferric Chloride, Ethylene diamine tetra- acetic acid (EDTA), Ferrous sulphate (FeSO<sub>4</sub>), Sodium nitroprusside, Sulphanilamide, Phosphoric acid, Hydrogen peroxide, Trichloroacetic acid, Serum albumin, 2deoxyribose, Hydrochloric acid were obtained from Sd fine Chemicals Ltd., India. Sodium azide, Nicotinamide adenine dinucleotide phosphate (NADPH), 5,5-Dithio-bis-(2-nitrobenzoic acid) (DTNB), Naphthyl ethylene diamine dihydrochloride, Glutathione oxidised (GSSG), Nicotine bi tartarate were obtained from Himedia Laboratories Pvt. Limited, Mumbai. 1-chloro-2,4,-dinitrobenzene (CDNB), Thiobarbituric acid were obtained from Rolex Chemical Industries, Mumbai. Reduced Glutathione (GSH), Sodium citrate, vitamin.E, Mannitol, Acetyl Salicylic acid, Epinephrine were obtained from, Loba Chemical Pvt. Limited. All other Chemicals and reagents used were of analytical grade.

#### In-vivo methods

### **Experimental animals**

Adult male wistar rats weighing 150-200 gms were used in the pharmacological studies. The inbred animals were taken from the animal house in Vel's College of Pharmacy, Pallavaram, Chennai -117. The animals were maintained in well-ventilated room temperature with natural day-night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai - 35, and tap water *ad libitum* throughout the experimental period. The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature.

The experimental protocol was approved by the Institutional Animal Ethics Committee IAEC Ref. No. 290/CPCSEA dated 21.12.04

### Preparation of drug solution

Nicotine bitartrate was dissolved in isotonic saline, and the pH was adjusted to 7 with NaOH solution<sup>(6)</sup>.

#### **Experimental design**

All the animals were divided into 5 groups containing six rats in each group. The number of correct responses before committing the first error was noted for all the rats. Then the animals were subjected to following drug treatment.

Group 1 served as control, received saline.

Group 2 animals received nicotine 0.2 mg/kg i.p. for 12 days.

**Group 3** animals received nicotine 0.8 mg/kg i.p. twice daily for 4 days

Group 4 received nicotine 0.1 mg/kg s.c. for 14 days.

**Group 5** received standard drug piracetam 250 mg/kg p.o. for 8 days.

The nootropic activity of nicotine was assessed using the following animal model

# 1. Step-down paradigm<sup>(7)</sup>

The animals were initially allowed to explore itself the surroundings of the box for 10 sec. Then the animals were placed on a wooden platform located in the center of the grid floor and latency time (step down latency SDL) to descend with all its paws to grid floor was noted. Initially electric current (1 mA) is passed through grid floor for one second. When animal descends down from the platform, step down latency as initial response was noted for all the animals. Then the animals were subjected to above stated drug treatment.

30 min after last injection animals were placed again on the platform and latency time to step-down was noted. Cut of time is fixed at 60 sec. Increase in step down latency time is considered as evidence for successful acquisition and retrieval of unpleasant experience.

# 2. Step-through paradigm<sup>(8)</sup>

The test apparatus consists of a small illuminated chamber connected to a large dark chamber through a guillotine door. Animals were placed in the illuminated compartment at the farthest point from the guillotine door. The latency time to enter the dark compartment was noted. If the animal does not enter the dark compartment within 180 sec, it is forcibly pushed to dark compartment. The door is shut automatically and an electric foot shock of 1 mA delivered for 1 sec. The animals were then quickly removed and returned to home cage. Latency time was noted for all the animals. The retention is evaluated after 24 hrs. Then the animals were subjected to the scheduled drug treatment.

30 min after last injection animals were placed again in illuminated compartment. The time taken to step from illuminated to

dark compartment was noted. Prolongation of this step through latency time indicates acquisition and retrieval of the foot shock received by the animals.

#### 3. Retrieval paradigm

This model is modified as trial to criteria inhibitory avoidance. In this modified version test animals were placed back in the illuminated compartment after subjecting the animals to foot shock in dark chamber and it is repeated till the animal remains in the illuminated chamber for 60 sec. The number of trials required to achieve this end point was noted and is considered to be an index of acquisition. The animals were subjected to scheduled drug treatment.

Retrieval is evaluated in the same manner and the number of trials required to achieve this end point was noted.

# 4. Radial arm maze<sup>(9)</sup>

At the beginning of a trial, food pellet was placed at the end of each arm of radial arm maze. The rat was placed in the central hub with all guillotine doors lowered. Then all the doors are simultaneously opened, to allow rat to choose arms freely. When the rat enters one of the arm, the doors to the remaining seven arms were closed. The animal returns to the central hub after eating the foot pellet placed at the end of that arm and then the door to that arm is also closed, confining the rat to the center of the hub. After a gap of 60 sec, all the doors were opened again. The trial was considered complete when the rat visits all eight arms. Re-entry to an arm is considered as an error. The number of correct responses before committing the first error (the number of initial correct responses) was calculated as the index of radial arm maze performance.



After drug treatment animals were again placed in a radial arm maze and number of correct responses for all the rats were noted. If the drug having memory enhancing activity, the rats show good performance in radial arm maze.

# Preparation of brain homogenate

Rats of all the groups after subjected to cognitive performance study, were then sacrificed by cervical decapitation under light ether anesthesia. Immediately after sacrifice, the brain was excised from the animals, washed in ice cold saline, 10% homogenate was prepared in the cold phosphate buffer (0.1 m, pH 7.4)<sup>(10)</sup>. The homogenate was centrifuged for 20 min at 4°C about 2000 rpm and the supernatant was used for the estimation of lipid peroxidation and cytoprotective enzymes, namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). All the estimations were done at particular nm using Shimadzu spectrophotometer, UV-1601 model.

# Estimation of superoxide dismutase

Superoxide dismutase was estimated according to the method of Misra and Fridovich <sup>(11)</sup>.

# Estimation of catalase

Catalase of brain homogenate was estimated according to the method of Bergmeyer et al.<sup>(12)</sup>

# Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase of brain homogenate was estimated according to the method of Necheles et al<sup>(13)</sup>.

# Estimation of glutathione reductase

Glutathione reductase activity of brain homogenate was measured by the method of Dobler et al  $^{(14)}$ .

# Estimation of lipid peroxidation

Lipid peroxidation in brain homogenate was assayed according to the method of Wills.<sup>(15)</sup>

#### In-vitro methods

**Effect of nicotine on hydroxyl radical scavenging** by the method of Ahakukakorn et al <sup>(16)</sup>

**Effect of nicotine on hydroxyl radical production** by the method of Kessler et al <sup>(17)</sup>

Effect of nicotine on  $Fe^{2+}$  / Ascorbate induced lipid peroxidation by the method of Mary et al<sup>(18)</sup>

**Effect of nicotine on protein denaturation** by the method of Chatterjee et al <sup>(19)</sup>

# Statistical analysis

Statistical significance tests for comparison were done by ANOVA, using Dunnet's t-test

#### Results

# Step down, Step through and Retrieval paradigm

All the nicotine treated groups (0.2 mg / kg i.p 12 days, 0.8 mg/kg twice daily for 4 days, 0.1 mg / kg s.c for 14 days) showed statistically significant increase in the latency time to step down (Fig. 1) and step through (Fig.2). There is also significant decrease in the number of trials required for retrieval paradigm, when comparing with control (Fig.3).

### **Radial Arm Maze**

As shown in the fig.4 all the nicotine treated (0.2 mg/ kg i.p for 12 days, 0.8 mg/kg i.p. twice daily for 4 days, 0.1 mg / kg s.c for 14 days) groups showed statistically significant increase in the number of initial correct responses, when compared with control group.

#### Effect of Nicotine on Cytoprotective enzymes

Results of table .1 indicate that nicotine treated rats (0.2 mg/kg i.p for 12 days, 0.8 mg/kg i.p. twice daily for 4 days, 0.1 mg/kg s.c for 14 days) showed slight decrease in the level of cytoprotective enzymes (SOD, CAT, GPx & GR) but it was not statistically significant when compared with the control group.

#### Effect of nicotine on Lipid Peroxidation

Lipid peroxide in brain homogenate of nicotine treated groups (0.2 mg /kg i.p for 12 days, 0.8 mg / kg twice daily for 4 days, 0.1 mg / kg s.c. for 14 days) showed slight increase (table 1) in peroxide level. But when compared with control group results are statistically not significant.

# OH<sup>•</sup> scavenging and OH<sup>•</sup> production

Results are shown in Fig.5. These results suggests that nicotine at all tested dose levels showed significant scavenging of OH<sup>•</sup> generated by the EDTA/H<sub>2</sub>O<sub>2</sub> system when compared with that of control. The percentage scavenging of OH<sup>•</sup> by nicotine increased

in a dose dependent manner. Results were comparable with standard (Mannitol 100 nM). It was also shown from **Fig.6**, nicotine (100 to 600 nM) was not able to generate hydroxyl radical in deoxyribose degradation assay method.

# Effect of nicotine on Fe<sup>2+</sup> / Ascorbate and vitamin-E induced lipid peroxidation.

Nicotine significantly (P<0.001) inhibited the peroxide formation in rat brain homogenate *in vitro*, at all tested dose levels (100 to 600 nM) when compared with that of control. The peroxide inhibition of nicotine increased in a dose dependent manner. Results were comparable with standard (vitamin-E 600 nM) (P<0.001). (Fig.7)

## Effect of nicotine on Protein denaturation

From Fig.8, it was observed that nicotine (100 to 600 nm) showed significant protection against protein denaturation in a dose dependant manner. Results were comparable with standard.





All the values are expressed as mean + SEM (n=6)

\*P < 0.001 when the comparison is made between before and after treatment latency time.



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Table.1: Effect of Nicotine on Brain cellular antioxidant enzymes and lipid peroxidation at different doses.

IV	Nicotine 0.1 mg/kg s c	$241.2 + 3.86^{NS}$	$3.2 + 1.65^{\rm NS}$	$13.06 + 1.36^{\rm NS}$	$16.3 + 1.17^{\rm NS}$	1.55+ 0.22 <sup>NS</sup>
III	Nicotine 0.8 mg/kg i n twice daily	$247.6 + 14.72^{\rm NS}$	$3.65 + 1.53^{\rm NS}$	12.95 + 1.48 <sup>NS</sup>	$16.4 + 1.31^{\rm NS}$	$1.54+ 0.34^{\rm NS}$
П	Nicotine 0 2mg/kg i n for 12	247.5 + 15.29 <sup>NS</sup>	$4.08 + 1.05^{\rm NS}$	$12.9 + 1.22^{\rm NS}$	$16.05 + 2.13^{\rm NS}$	$1.54 + 0.28^{\rm NS}$
Ι	Control (Saline)	250.7 + 14.31	4.78 + 2.39	13.41+ 1.07	16.33 + 1.05	1.50 + 0.16
	GROUP	SOD levels in brain homogenate	CAT levels in brain homogenate	GPx levels in brain homogenate	GR levels in brain homogenate	LPO levels in brain homogenate

All the values are expressed as mean + SEM (n=6) \* p<0.001 when test and standard is compared against control NS - Non-significant Enzyme units are expressed as: SOD - unit /mg protein x  $10^{-3}$ CAT - n moles of H<sub>2</sub>O<sub>2</sub> utilised /min/mg protein GP<sub>x</sub> - n moles of GSH oxidised/min/mg protein GR - n moles of GSSG utilized/min/mg protein LPO - n moles of MDA liberated/min/mg protein Fig.5



Effect of Nicotine on Hydroxyl radical scavenging activity

Values are mean + SEM of 6 replicates

\* p<0.001 When test and standard are compared against control.





**OH**<sup>•</sup> Production

Values are mean + SEM of 6 replicates

\* p<0.001 When test and standard are compared against control.



Values are mean + SEM of 6 replicates

\* p<0.001 When test and standard are compared against control.



Values are mean + SEM of 6 replicates

\* p<0.001 When test and standard are compared against control.

# Conclusion

The present article consists of a complete survey of cognitive performance of nicotine, along with its pro-oxidant / antioxidant properties in the periphery and in CNS.

Nootropic activity of nicotine was conducted using rat models. There are strong evidences that nicotine enhances cognitive performance both in animal models and human subjects.

Free radicals and other reactive species have been implicated in the progression of at least 100 different diseases, so the interest in free radicals and oxidative stress has grown. It is highly debatable whether nicotine deleterious effects in CNS are due to free radical production: particularly, because evidence has shown nicotine to have both antioxidant effects and pro-oxidant effects. As it is a matter of importance, the antioxidant and pro-oxidant effect of nicotine in CNS study was designed.

Both the *in vivo* and *in vitro* models were used for the study. So far very few studies have been examined the effect of nicotine on CNS in relation to oxidative stress. Those studies employed higher concentrations of nicotine and failed to assess lipid peroxidation, antioxidant enzymes and indicators of ROS production. In our present investigation we employed relevant concentration of nicotine (proven to improve cognitive performance both clinically and by animal models) to measure lipid peroxidation and antioxidant enzyme levels in brain homogenate. These dose levels of nicotine neither depleted nor improved antioxidant enzyme levels. There was also no increase in the peroxide formation.

Our *in vitro* results also suggested nicotine's iron binding capacity, OH scavenging and protection against protein denaturation effects.

In conclusion, the studies presented in this thesis indicate that under certain circumstances nicotine shows antioxidant properties.

Still, the questions remain has to whether nicotine may have intracellular antioxidant properties influenced by activation of the nicotinic receptor; and whether it may contribute to extracellular oxidative stress through reaction with endogenous substances and / or disruption of the cellular membrane. Further studies examining these properties of nicotine need to be more specific in determining true mechanism of action. Also, in addition, no study to date has used human-derived cell lines to examine the oxidative stress or antioxidant properties of nicotine. Given the differences between human and rodent physiology and anatomy, further studies that employ human cell lines would be on interest and may help in determining the properties of nicotine in vitro.

Hence there is still need for research that will resolve prooxidant or antioxidant role of nicotine and the nicotinic receptor in the brain and behaviour.

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