



Long Term Administration of Nicotine in Albino Rat Cerebellum - Histopathological Study

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ABSTRACT

The actions of nicotine upon cerebellar function are poorly understood. Small doses of nicotine have a stimulating action on the central nervous system whereas large doses depress. 16 adult male albino rats of wistar strain weighing 135-150 grams were used for the present study. The rats were divided into four groups each consists of 4 animals. The control group was given 0.9% saline as vehicle and the experimental groups (drug treated- II, III, IV) were given intraperitoneal injection of Nicotine a dose of 0.5 mg/kg, 0.75 mg/kg and 1mg/kg body weight dissolved 1:1 ratio in normal saline respectively for a period of 28 days. Cortical layers were intact in the entire drug treated group. White matter with few vacuoles was observed in 0.5 mg/kg Nicotine induced group. Disruption of Purkinje cell layer and granular cells was observed in 0.75 mg/kg induced group. Dose of 1mg/kg nicotine induced group animals shown complete disruption of Purkinje cell layer, cellular disruption in granular cell layer. Extended core of white matter into the folia of the cerebellum cellular microcystic change with interstitial oedema was observed in the same group. The loss of white core, Purkinje cell layer, and granular layer cellular disruption give better knowledge in understanding long term nicotine exposure. Our study reveals the histopathological changes of nicotine on long term administration in adolescent rat cerebellum with their clinical significance.

Keywords: cerebellum, disruption, nicotine, white core.

INTRODUCTION

Recent studies on neurophysicochemical aspects also suggested that the cerebellum also could participate in excitotoxicity. Dysfunction of the cerebellum causes problems such as loss of balance and motor coordination, ataxia, dystonia through several genetic mutations and alterations in the biochemical pathways of neuroprotection; however, a large number of patients remain undiagnosed.¹ Many researchers have studied the effect of nicotine on cerebellum and found significant deficits.² Nicotinic receptor binding sites and subunits are expressed in rat cerebellum from embryonic stage through adult hood, with the highest expression levels seen during the development of the cerebellar cortex.^{2,3} Nicotine acts as neurodegenerative agent on higher doses, recent studies stating that nicotine can use as protective agent due to its positive effects in the same area but still its action of mechanism unknown.

MATERIALS AND METHODS

A total of 16 adult male albino rats of wistar strain weighing 135-150 grams were used for the present study. The animals were maintained under controlled conditions and in room temperature (23±2° C), humidity (50±5%) and a 12 h light and dark cycle. The animals were housed in sanitized polypropylene cages. The animals were fed with standard rat pellet diet commercially available manufactured by Kamathenu Pvt Ltd., Bangalore and

clean drinking water ad libitum. The rats were divided into four groups each consists of 4 animals.

The control group was given 0.9% saline as vehicle and the experimental groups (drug treated-II,III,IV) were given an intra peritoneal injection dose of 0.5 mg/kg, 0.75 mg/kg, 1mg/kg body weight Nicotine dissolved in normal saline (0.9% NaCl) respectively for a period of 28 days.

All the animals were sacrificed after a 12 hrs of fasting from the last day of experimental study and preserved in the 10% formalin for 48 hours.

The specimens were processed for routine paraffin technique to study the histopathological changes in cerebellum on administration of nicotine.

All the animals were maintained as per the national guidelines and protocols approved by Institutional animal ethical committee of Sugan Life sciences (SLS/COM/IAEC/02/2013-2014).

RESULTS

Molecular layer with basket and stellate cells, single Purkinje cell layer lies between the molecular and granular cell layer was observed in control group. We have observed Column of nerve fibers in the white matter of cerebellum. Granular cell layer with granule cells (pink stained) glomeruli (light stained) were also observed in control group (Figure - 1).



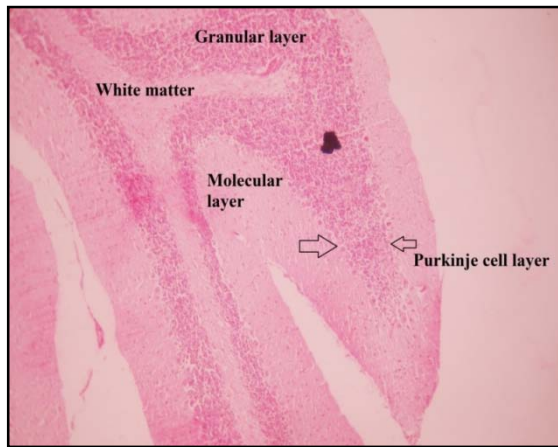


Figure 1: Cortical layers of cerebellum and white matter in the control group (10X)

In 0.5 mg /kg nicotine treated Group (II); there was vacuoles in the white matter and all the layers of cortex were observed (Figure - 2).

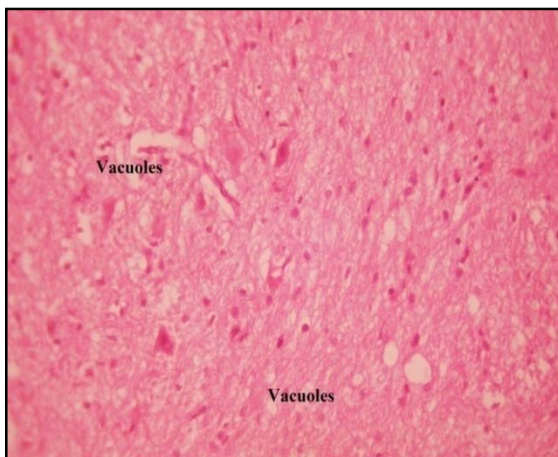


Figure 2: Vacuoles in the white matter was observed in animals treated with 0.5mg/kg Nicotine (40X)

Disruption of purkinje cells layer and granular cells was observed in 0.75 mg/kg nicotine treated group (III). We were also observed few purkinje cells in the same group (Figure-3).

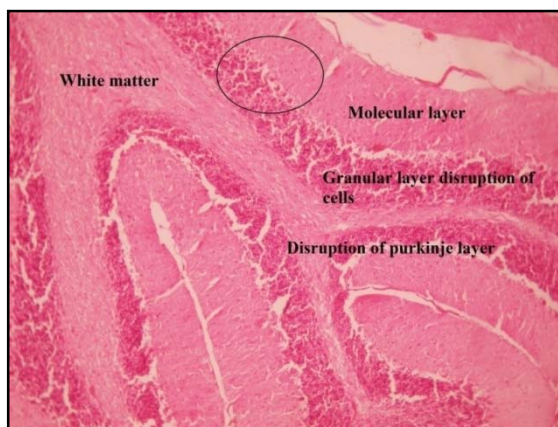


Figure 3: Disruption of purkinje cells layer and granular cells was observed in animals treated with 0.75mg/kg Nicotine (10X) [circle-purkinje cells]

Vacuoles in the white matter and disruption of cells in the granular layer under high magnification were observed (Figure-4).

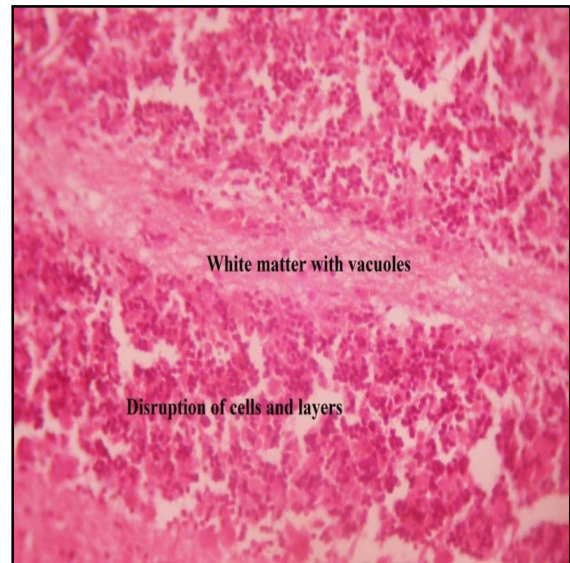


Figure 4: Vacuoles in the white matter and disruption of cells in the granular layer in animals treated with 0.75mg/kg Nicotine (40X)

In 1mg /kg nicotine treated group (IV), there was complete disruption of Purkinje cell layer along with a loss of Purkinje cell layer (very few cells only present) and also cellular disruptions in granular cells were observed (Figure-5).

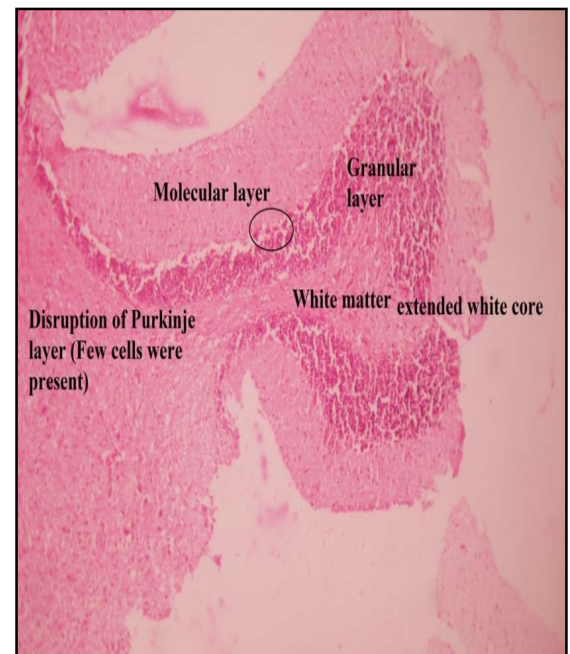


Figure 5: Complete disruption of Purkinje cell layer, loss of Purkinje cell layer (very few cells only present) and also cellular disruption in granular cells in animals treated with 1mg/kg Nicotine (10X) [circle-purkinje cells]

Under high magnification we have observed the extended core of white matter into the folia of the cerebellum, cellular microcystic change with interstitial oedema of

white matter and significant loss of Purkinje cells with disruption of cellular layers were observed (Figure - 6).



Figure 6: Extended core of white matter into the folia of the cerebellum with cellular microcystic change with interstitial oedema in animals treated with 1mg/kg Nicotine (40X) [circle - purkinje cells]

DISCUSSION

The interaction of nicotine and the Alpha subunits of nicotine Ach receptor in white core may subsequently trigger apoptotic process that leads to loss of white matter. In our study there was a loss of white core of cerebellum on long term administration of nicotine.^{4,5} Adolescent nicotine exposure has deleterious effects on cell development particularly in Purkinje cell layer in cerebellum of albino rats characterized by reduced number of Purkinje cell number.⁶ Exposure to nicotine during the brain growth spurt period in rats has been associated with significant reduction of the Purkinje cell numbers in the cerebellar vermis.^{6,7} Belluzzi and co-workers reported that the brain in early adolescent, late adolescent and adult rats is differentially sensitive to the nicotine administration. The adolescent or adult brain could be more sensitive to acute and also to chronic administration of nicotine.^{8,9} Neuro-pathologically, reduction in Purkinje cell numbers in cerebellar vermis and hemispheres have been reported in nearly all mentally retarded autistic cases.^{10,11} The loss of Purkinje cells combined with loss of granule cells and white matter of cerebellum in two mentally retarded cases suggested that cerebellar hyperplasia.¹² On higher doses of nicotine for long term administration we have found Purkinje cell layer and granular cells disruption and loss which were in agreement with previous literatures.¹³ One-dose application had no significant effect, but the chronic treatment was accompanied with a reduction in locomotor activity in adult rats.¹⁴

Administration of nicotine specifically inhibits cerebellar Purkinje cells which could result in the reduction of Purkinje cells, and consequently, the middle Purkinje cell layer of the cerebellum.

The results and observations of our study were in agreement with literature.¹⁵

CONCLUSION

The loss of white core, Purkinje cell layer, and granular layer cellular disruption give better knowledge in understanding long term nicotine exposure in animal models.

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REFERENCES

1. Tsugunobu A, Hiroyuki K, Kazumi M, Kenji N, Yasushi K, Tsushi M. Protective effect of IL-18 on Kainate- and IL-1 β -induced cerebellar ataxia in mice. *Journal of Immunology*, 180, 2008, 2322-2328.
2. Tewari A., Hasan M., Sahai A., Sharma P.K., Rani A., Agarwal A.K. White core cerebellum in nicotine treated rats: a histological study. *J. Anat. Soc. India*, 59(2), 2010, 150-153.
3. G. De Filippi, T. Baldwinson, E. Sher. Nicotinic receptor modulation of neurotransmitter release in the cerebellum. *Prog Brain Res*. 148, 2005, 307-20.
4. Prendergast M A, Harris B R, Mayer S, Nicotine exposure reduces N-methyl-D-aspartate toxicity in the hippocampus relation to distribution of the alpha 7 nicotinic acetylcholine receptor subunit. *Med. Sci. Monit*. 7, 2001, 1153-1160.
5. Wright S C, Zhong J, Zheng H. Nicotine inhibition of apoptosis suggests a role in tumor production. *Faseb J*. 7, 1993, 1045-1051.
6. Chen A, Russell B. Edwards, Ronald D. Romero, Long term nicotine exposure reduces Purkinje cell number in the adult rat cerebellar vermis. *Neurotoxicology & Teratology*. 25, 2003, 325-334.
7. Anshu Tewari, Mahdi Hasan, A. Sahai, P. K. Sharma, A. K. Agarwal. Nicotine mediated microcystic oedema in white matter of cerebellum: possible relationship to postural imbalance. *Annals of Neurosciences*. Vol. 18(1), 2011, 14-17.
8. Belluzzi J. D., Lee A. G., Oliff H. S. Age-dependent effect of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology*. 174, 2004, 389-395.
9. Pereira M, Strupp T, Holzleitner T, Smoking correlation of nicotine induced nystagmus & postural disbalances. *Neuroreport*. 8, 2001, 1223-1226.
10. Ritvo E. R., Freeman B. J., Scheibel A. B., Duong T., Robinson H., Guthrie D., Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC autopsy research report. *Am. J. Psychiatry*. 143, 1986, 862-866.
11. Bailey A., Luthert P., Dean A., Harding B., Janota I., Montgomery M., A clinicopathological study of autism. *Brain*. 121, 1998, 889-905.

12. Bauman M. L., Kemper T. L. Histoanatomic observations of the brain in early infantile autism. *Neurology*. 35, 1985, 866–874.
13. M. Lee, C. Martin-Ruiz, A. Graham, J. Court, E. Jaros, R. Perry, P. Iversen, M. Bauman, E. Perry. Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain*. 125(7), 2002, 1483-1495.
14. Slawecki C. J., Ehlers C. L. Lasting effects of adolescent nicotine exposure on the electroencephalogram, event related potentials, and locomotor activity in the rat. *Brain Res. Dev. Brain Res*. 138, 2002, 15–25.
15. De la Garza R., Freedman R., Hoffer J. Nicotine-induced inhibition of cerebellar Purkinje neurons: specific actions of nicotine and selective blockade by mecamylamine. *Neuropharmacology*. 28(5), 1989, 495-501.

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