Hippocampal and striatal histomorphology following chronic nicotine administration in female and male rats

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KEY WORDS

Nicotine
Hippocampus
Striatum
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Males

ABSTRACT

Background: Nicotine is a subject of continuous research because of its likely ameliorative effects on neurologic and neurodegenerative disorders.
Purpose: This study examined the effects of its chronic subcutaneous administration on hippocampal and striatal microstructure in both female and male rats.
Methods: Forty adult female and male Wistar rats were divided into 4 groups. Three experimental groups were administered nicotine via subcutaneous injections at doses of 0.25, 2 and 4 mg/kg body weight for 28 days. Control groups received normal saline. Following administration, routine processing of brain tissues was carried out. Sections obtained were stained using routine H&E methods for general histological appearance, Cresyl violet methods for nissl substances, and Bielschwolsky silver impregnation method for neuritic plaques and neurofibrillary tangles.
Results: The study showed significant increase in percentage of neurons showing degenerating features in the hippocampus and striatum of both female and male rats following the higher doses of nicotine treatment. Only female rats showed positive agyrophilic (black-coloured) aggregations in the hippocampal and striatal regions after nicotine treatment.
Conclusion: The present study indicates that higher dose of chronic nicotine administration may induce hippocampal and striatal neurodegenerative changes. However, further studies using more specific method for studying neurodegeneration within brain regions is recommended.

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Introduction

Nicotine, in its unadulterated form is odourless and only takes the distinctive whiff of tobacco after exposure to air. It is considered to be the most widely used stimulant next to caffeine.[1] It is prone to varying grades of abuse. It may be used nonchalantly or with the same uncontrollability as other communally unaccepted drug, particularly via smoking.[1] Nicotine is also readily available in various forms of nicotine replacement therapy such as transdermal patches and nicotine chewing gums. Nicotine is the main component of tobacco smoke. After inhalation, it is distributed through the blood stream and can cross the blood-brain barrier. It takes only a few seconds to reach the brain.[2,3] Nicotine acts on the neuronal nicotinic acetylcholine receptors (nAChR), located on cholinergic synapses in the peripheral and central nervous system.[4] Nicotine has been considered to have many adverse effects, but in the last decade it has been the subject of potential therapeutic value for the management of neurologic and neurodegenerative diseases.[5,6] Hence, research on the effects of nicotine in the body, especially on the central nervous system are continuous. The present study examined the effects of its chronic subcutaneous administration on hippocampal and striatal microstructure in both female and male rats.

Methods

Animal Care and Drug administration

Forty adult Wistar rats (150–200 g) were used for this study. There were 20 females and 20 males rats. The rats were randomly divided into 4 groups of 5 females and 5 males. Experimental groups were administered nicotine via subcutaneous injections at 0.25, 2 and 4 mg/kg body weight for 28 days. Normal saline was administered to control rats. The selection of nicotine dose is based on previously published studies.[7–9]

Animals were given standard laboratory rat chow and water *ad libitum*. All rats were handled in accordance with the guidelines for animal research as detailed in the Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health, 2011). Nicotine was obtained in free base form as (–)-Nicotine.

Histological studies

Following administration, rats were euthanized and the brain excised. Fixation of brain tissues was by immersion in 10% neutral buffered formalin. Following fixation, tissues were processed for rapid routine tissue processing, and serial sections were obtained on a rotary microtome at 6 μm thickness. Tissues were stained as previously described using Haematoxylin and Eosin (H&E) methods for general histological appearance;[10] Cresyl violet methods for nissl substances;[11] and Bielschwolsky silver impregnation method for neuritic plaques and neurofibrillary tangles.[12,13]

Photomicrography and Image Analysis

Stained sections were examined using a Leica DM750 digital bright-field microscope and digital photomicrographs taken with the aid of attached Leica ICC50 camera. Micrographs of H&E sections were imported into Image J software

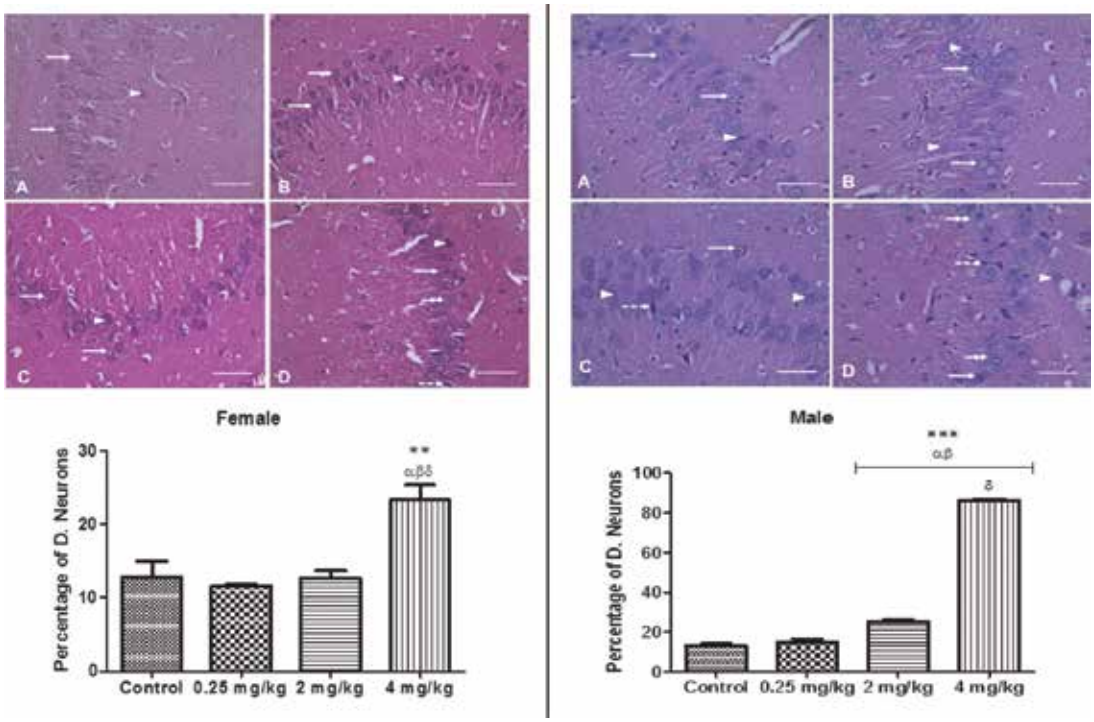

Fig. 1: *Upper panel* – Micrographs of hippocampus (CA3) of control and nicotine treated male and female rats. Observe the intact pyramidal neurons (arrows) with large nuclei and prominent nucleoli. Also seen the different characteristic features of degenerating neurons; prominent eosinophilic cytoplasm with or without shrunken nuclei (arrow heads), pyknotic nuclei (dashed arrows), neuron swelling and/or vacuolation within the cytoplasm (double arrows). H&E, Scale bars –50 μm. *Lower panel* – Image J neuronal counting of H&E stained sections of the Hippocampus (CA3). D. Neurons denote degenerating neurons. Values are expressed as mean ± SEM. **p<0.01, ***p<0.001. α, β, and δ denote significant difference compared to group Control, 0.25 mg/kg and between 2 and 4 mg/kg respectively. One way ANOVA followed by Student Newman-Keuls (SNK).

(NIH-sponsored public domain image analysis software). Intact neurons and those showing degenerating features were identified and counted using the Image J cell counter plugin. software. Percentage of neurons showing degeneration features were expressed as: (Number of neurons showing degenerating features/Total number of neurons) x 100.

Statistical analysis

Data were analysed using One-way ANOVA, followed by Student Newman-Keuls (SNK) test for multiple comparisons, and expressed as mean ± SEM. GraphPad Prism 5 (San Diego, USA) was the software used for data analysis. P<0.05 was taken as significant difference.

Results

Features of degenerating neurons, as can be seen in light microscopy study of H&E stained sections, were increasingly observed in hippocampus and striatum following the higher doses of nicotine administration to both female and male groups. These features include prominent eosinophilic cytoplasm with or without shrunken nuclei, pyknotic nuclei, and neuronal swelling and/or vacuolation within the cytoplasm.[14,15] Image J analysis, revealed that in the hippocampus, there was significant increase ($p<0.01$) in percentage of neurons showing degenerating features only with 4 mg/kg nicotine treatment in female rats. However, in male rats, both 2 and 4 mg/kg treatment significantly increased ($p<0.001$) percentage of neurons showing degenerating features compared to control and 0.25 mg/kg treatment. Though, 4 mg/kg treatment resulted in significantly higher percentage compared to 2 mg/kg treatment (Fig. 1). On the other hand, analysis of sections of the striatum revealed that 2 and 4 mg/kg treatment significantly increased ($p<0.001$) percentage of neurons showing degenerating features compared to control and 0.25 mg/kg treatment for both female and male rats. Though, only in male rats did 4 mg/kg treatment result in significantly higher percentage compared to 2 mg/kg treatment (Fig. 2).

Sections stained with Cresyl violet revealed no observable differences in intensity of hippocampal and striatal nissl substances following nicotine administrations in female rats. However, male rats showed better intensity of hippocampal nissl substances following 0.25 mg/kg but no observable difference in the striatum (Figs. 3 and 4, Table 1).

Bielschwolsky silver impregnation method showed positive agyrophilic (black-coloured) aggregations in the hippocampal and striatal regions of nicotine treated female rats. These are suggestive of neuritic plaques and neurofibrillary tangles formation. However, such agyrophilic (black-coloured) aggregations were absent in both the hippocampus and striatum of male rats (Figs. 5 and 6).

Discussion

The result of the present study indicates that chronic nicotine administration increased number of neurons showing degenerating features in the hippocampus and striatum at higher doses of 2 and 4 mg/kg treatment. Results suggest that 4 mg/kg treatment consistently increased degenerating

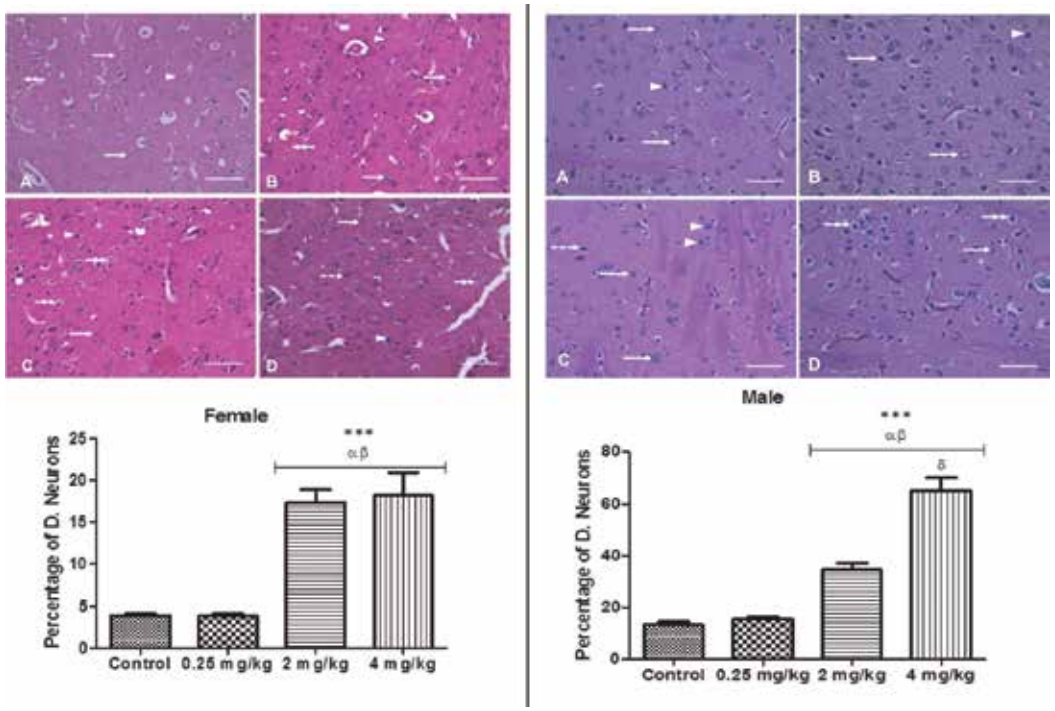

Fig. 2: *Upper panel* – Micrographs of striatum (Caudate-Putamen) of control and nicotine treated male and female rats. The intact neurons (arrows) can be obtained with rounded nuclei and prominent nucleoli. Also observe the different characteristic features of degenerating neurons; prominent eosinophilic cytoplasm with or without shrunken nuclei (arrow heads), pyknotic nuclei (dashed arrows), neuron swelling and/or vacuolation within the cytoplasm (double arrows). H&E, Scale bars –50 μm. *Lower panel* – Image J neuronal counting of H&E stained sections of the striatum (Caudate-Putamen). D. Neurons denote degenerating neurons. Values are expressed as mean ± SEM. ***p<0.001. α, β, and δ denote significant difference compared to group Control, 0.25 mg/kg and between 2 and 4 mg/kg respectively. One way ANOVA followed by Student Newman-Keuls (SNK).

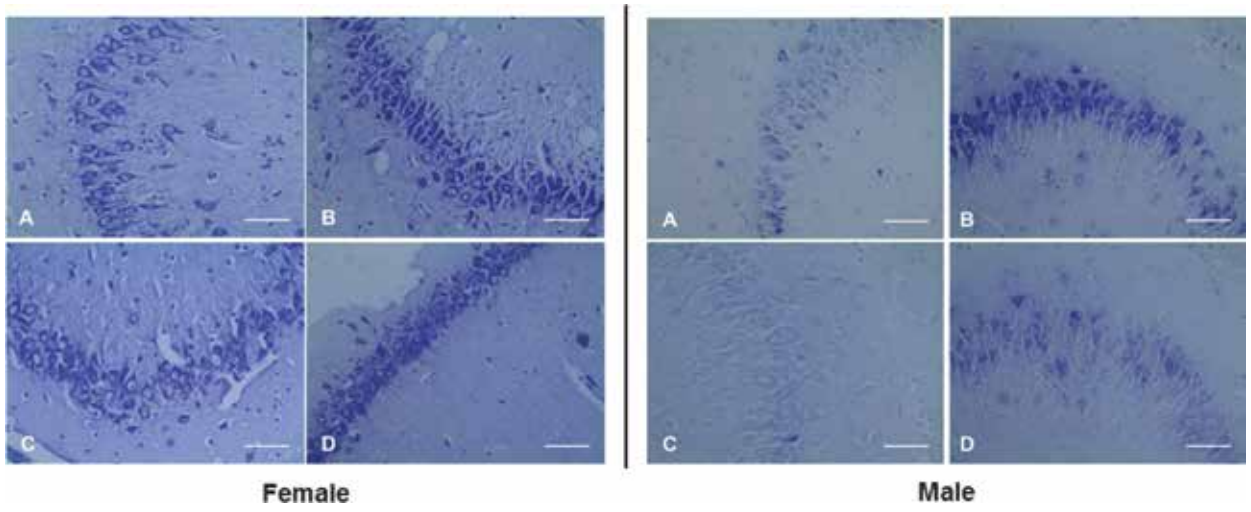

Fig. 3: Micrographs of hippocampus (CA3) of control and nicotine treated male and female rats. Cresyl violet, Scale bars –50 μm.

features in hippocampus and striatum of both female and male rats.

Higher doses of nicotine have previously been shown to produce selective degeneration in the brain. A study has shown that nicotine could damage the adult cerebellum by depleting the white core of the cerebellum, at high doses of 5 and 10 mg/kg administered for 60 days.[16] However, Carlson et al., observed that high doses of 15, 13, 7 and 3 mg/kg administered for 5 days produced degeneration that was specific to axons of the fasciculus retroflexus (FR); a major pathway for dopaminergic targets and other limbic structures onto important midbrain targets such as substantia nigra and ventral tegmental area.[17] The axons of FR represent a weak link subject to

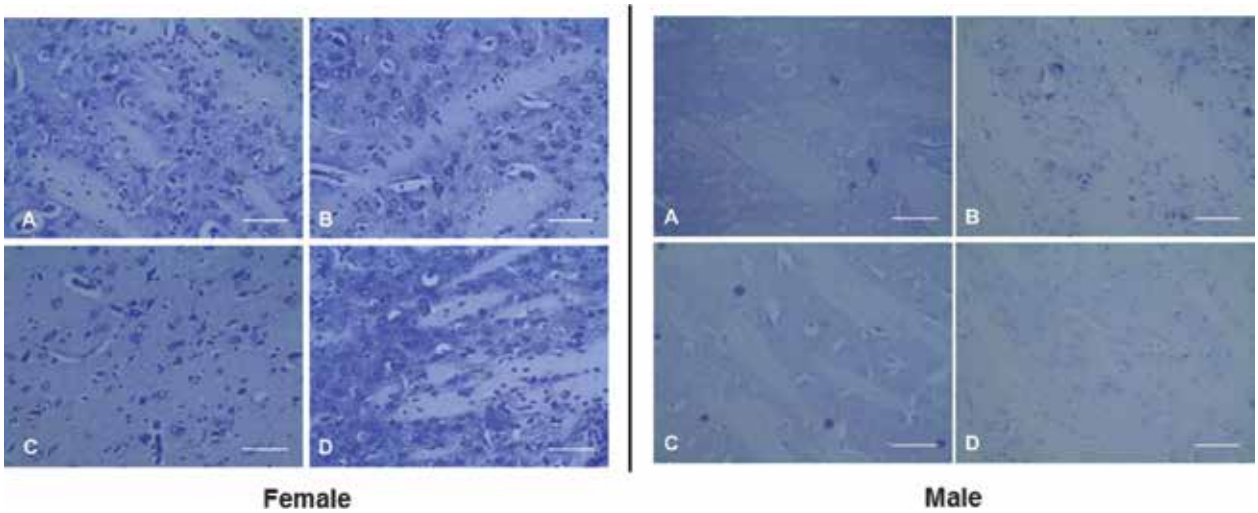

Fig. 4: Micrographs of striatum (Caudate-Putamen) of control and nicotine treated male and female rats. Cresyl violet, Scale bars –50 µm.

Table 1: Effect of nicotine administration on nissl substances staining

Nissl substances intensity				
	Hippocampal		Striatum	
	Female	Male	Female	Male
Control	++	+–	++	+–
0.25 mg/kg	++	++	++	+–
2 mg/kg	++	+–	++	+–
4 mg/kg	++	+–	++	+–

Notation: Intense: ++
Moderate/mild: + –

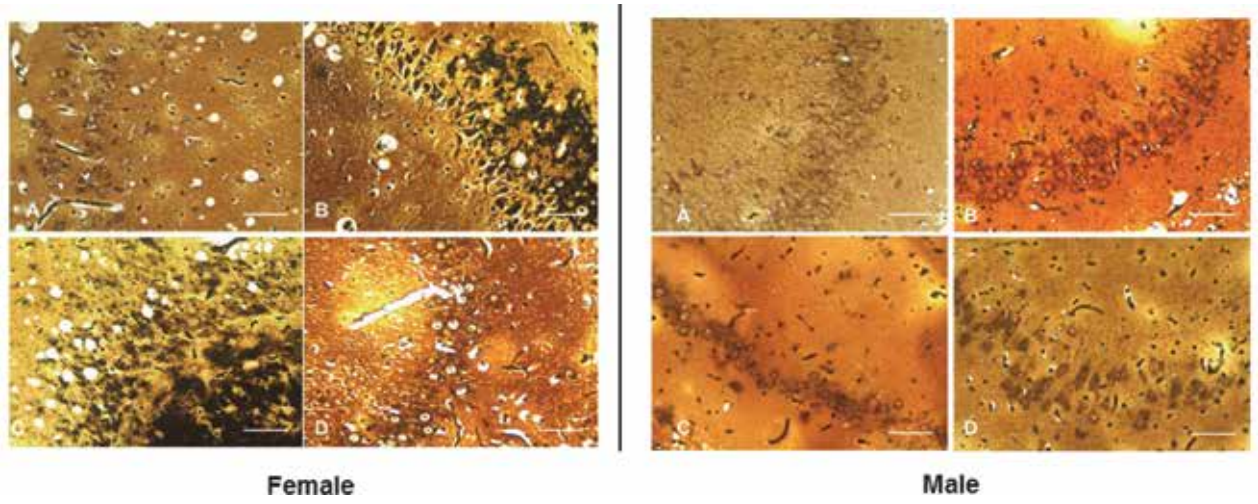

Fig. 5: Micrographs of hippocampus (CA3) of control and nicotine treated male and female rats. Observe agyrophilic (black-coloured) aggregations in nicotine treated female rats. Bielschwolsky staining, Scale bars –50 µm.

degeneration by major drugs of abuse including cocaine and amphetamines.[17,18] However, low dose of 1.7 mg/kg nicotine treatment for 5 days produced no such degenerative changes.[17] In view of these, the increase in degenerating features in the hippocampus and striatum as shown in the present study may represent significant neurodegeneration in these brain regions.

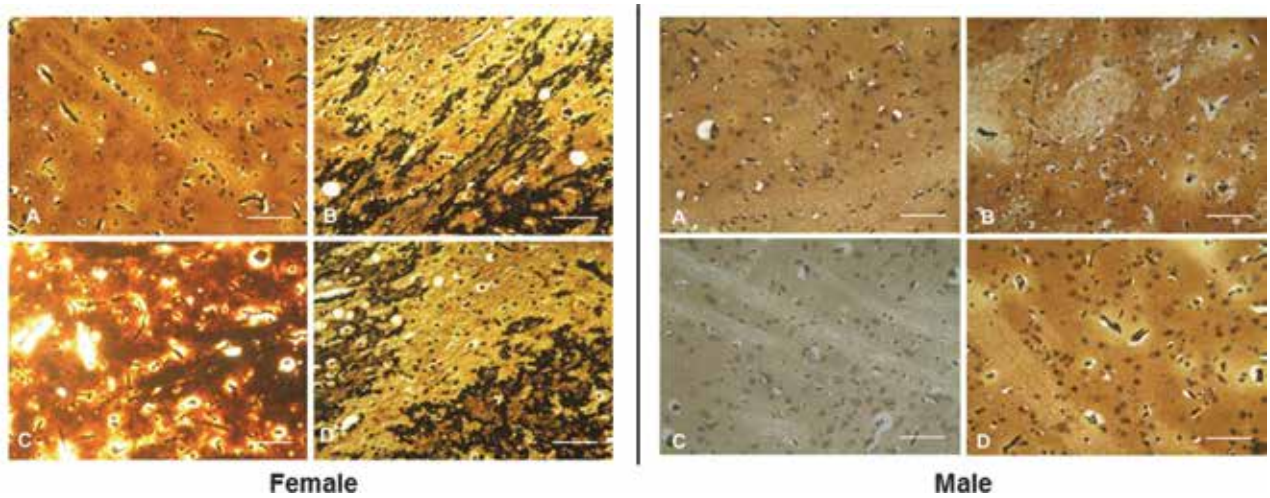

Fig. 6: Micrographs of striatum (Caudate-Putamen) of control and nicotine treated male and female rats. Observe agyrophilic (black-coloured) aggregations in nicotine treated female rats. Bielschwolsky staining, Scale bars –50 μm.

Nissl substances staining in the brain regions studied, showed no observable differences in both female and male rats compared to their corresponding controls, except in hippocampus of male rats following 0.25 mg/kg treatment. Increase in nissl substance staining at low dose in the male hippocampus may be suggestive of enhanced synthesis of intercellular proteins, as nissl substances represent rough endoplasmic reticulum within cells.[19–20] It is also important to note that only female rats showed features suggestive of neuritic plaques and neurofibrillary tangles formation. This alludes to the fact that the female brains may be more prone to negative effects of nicotine administration when such exposure takes place. In fact, reports have previously suggested that when nicotine does produce negative effects on brain and behaviours, females show more debilitating changes than males.[21] Although certain studies indicate that brain injury caused by other chemicals like scopalmine, diazapem, and omega N-nitro-L-arginine (LNNA) have more deteriorating effect on male mice,[22–24] the present study possibly indicate nicotine to be specifically hampering female brain activity.

In conclusion, the present study indicates that higher doses of chronic nicotine administration may induce neurodegenerative changes in the hippocampal and striatal brain regions. Some behavioural data (not included here) from this study did not show detrimental effects of nicotine on behaviours associated with the hippocampus and striatum. Thus we suggest that degenerating features seen may not be damaging enough to produce consequential changes in behaviour. In view of these we recommend further studies using more specific method for studying neurodegeneration within brain regions such as the fluoro-Jade-C staining.

Authorship contribution

Omamuyovwi M Ijomone: Involved in conception and study design, animal care and treatment, histological studies, image and data analysis, **Olayemi K Olaibi:** Involved in animal care and treatment, histological studies, image and data analysis, **Ugochukwu G Esomonu:** Involved in animal care and treatment, histological studies, **Polycarp U Nwoha:** Involved in conception and study design. All authors read and approved the final manuscript.

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References

1. Effraim KD, Modu S, Hamzah HG. Effect of crude garlic extract on nicotine induced hyperglycaemia and hyperlipidemia in rats. Afr J Biomed Res. 2000; 3: 125–127.
2. Hralova M, Maresova D, Riljak V. Effect of the single-dose of nicotine-administration on the brain bioelectrical activity and on behaviour in immature 12-day-old rats. Prague Med Report. 2010; 111: 182–190.
3. Benowitz NL, Hukkanen J, Jacob P. Nicotine chemistry, metabolism, kinetics and biomarkers. In: Nicotine Psychopharmacology, Handbook of Experimental Pharmacology, eds. Berlin Heidelberg: Springer-Verlag. 2009; 29–60.
4. Barik J, Wonnacott S. Molecular and cellular mechanisms of action of nicotine in the CNS. In: Nicotine Psychopharmacology, Handbook of Experimental Pharmacology, eds. Berlin Heidelberg: Springer-Verlag. 2009; 173–207.
5. Toyoshi U. Unusual effects of nicotine as a psychostimulant on ambulatory activity in mice. Int. Sch. Res. Net. (ISRN) Pharm 2012; Article ID 170981.
6. Yu W, Mechawar N, Krantic S, et al. α7 Nicotinic receptor activation reduces β-amyloid-induced apoptosis by inhibiting caspase-independent death through phosphatidylinositol 3-kinase signaling. J Neurochem. 2011; 119: 848–858.
7. Tariq M, Haseeb AK, Ibrahim E, et al. Neuroprotective effect of nicotine against 3-nitropropionic acid (3-NP)-induced experimental Huntington's disease in rats. Brain Res Bull. 2005; 67: 161–168.
8. Jain A, Flora SJS. Dose related effects of nicotine on oxidative injury in young, adult and old rats. J Environ Biol. 2012; 33: 233–38.
9. Scerri C. Effects of nicotine administration on amyloid precursor protein metabolism, neural cell genesis and acquisition of spatial memory. Malta Med J. 2011; 23: 1–9.
10. Ijomone OM, Obi AU. Kolaviron, isolated from Garcinia kola, inhibits acetylcholinesterase activities in the hippocampus and striatum of Wistar rats. Annals of Neurosci. 2013; 20(2); 2–8.
11. Nwoha PU, Ojo GB, Ajayi SA, et al. Garcinia kola diet provides slight neuroprotection to mice hippocampal neurons against neurotoxin. J Environ Neurosci Biomed. 2007; 1(2): 125–136.

12. Mirra SS, Hart MN, Terry RD. Making the diagnosis of Alzheimer's Disease. Arch Pathol Lab Med. 1993; 117: 132–144.
13. Ogundele OM, Enuaibe BU, Igwe J, et al. Histochemical investigation of acetyl cholinesterase activity and amyloid plaques in the visual relay centers: An approach in determining the mode of neuronal cell death in adult Wistar rats. J Clin Pathol Forensic Med. 2012; 3(1): 1–8.
14. Garman HR. Histology of the central nervous system. Toxicol Pathol. 2011; 39: 22–35.
15. Carliss RD, Radovsky A, Chengelis CP, et al. Oral administration of dextromethorphan does not produce neuronal vacuolation in the rat brain. NeuroToxicol. 2007; 28: 813–818.
16. Tewari A, Hasan M, Sahai A, et al. White core of cerebellum in nicotine treated rats- a histological study. J Anat Soc India. 2010; 59(2): 150–153.
17. Carlson J, Armstrong B, Switzer RC III, et al. Selective neurotoxic effects of nicotine on axons in fasciculus retroflexus further support evidence that this is a weak link in brain across multiple drugs of abuse. Neuropharmacol. 2000; 39: 2792–2798.
18. Carlson J, Noguchi K, Ellison G. Nicotine produces selective degeneration in the medial habenula and fasciculus retroflexus. Brain Res. 2001; 906: 127–134.
19. Herdegen T, Delgado-Garcia J. Brain damage and repair: from molecular research to clinical therapy. Springer. 2005; 37.
20. Thompson RH. The brain: a neuroscience primer. Worth Publishers. 2000; 35.
21. Robinson JD, Cinciripini PM, Tiffany ST, et al. Gender differences in affective response to acute nicotine administration and deprivation. Addictive Behav. 2007; 32: 543–561.
22. Saraf MK, Prabhakar S, Khanduja KL et al. Bacopa monniera Attenuates Scopolamine-Induced Impairment of Spatial Memory in Mice. Evid Based Complement Alternat Med. 2011; 2011: 236186.
23. Prabhakar S, Saraf MK, Banik A et al. Bacopa monniera selectively attenuates suppressed Superoxide dismutase activity in Diazepam induced amnesic mice. Ann Neurosci. 2011; 18(1): 8–13.
24. Anand A, Saraf MK, Prabhakar S. Antiamnesic effect of B. monniera on L-NNA induced amnesia involves calmodulin. Neurochem Res. 2010; 35 (8): 1172–81.