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Histomorphological Effects of Nicotine on Selected Parts of the Brain of Adult Wistar Rats

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Abstract. **Introduction**: Nicotine has been defined as a potent parasympathomimetic alkaloid that accumulates in the roots and leaves of Nightshade family of plants **Aim:** This study was aimed at evaluating the effects of orally ingested nicotine in the histology of hippocampus, substantia nigra and cerebellum.

Materials and Methods: Twenty four adult male Wistar rats (100g – 200g) were randomly divided into 4 groups (group 1 – group 4). Group 1 served as the control group, while groups 2 - 4 were the treated groups. Nicotine was diluted in water and 1ml of the different dosage (2mg/kg/day, 4mg/kg/day and 6mg/kg/day) were administered to the treated groups respectively with the aid of orogastric cannula for 42 days. Animals were euthanized by cervical dislocation at the end of 7, 21 and 42 days so as to demonstrate the dose and time dependant effect of this agent. Brain tissues were harvested, processed and stained using Haematoxylin and eosin according to standard histological techniques. Stained tissue images were captured using digital micrometer eyepiece and cell count was determined using stereological technique.

Statistical analysis: Data obtained were subjected to statistical analysis with the use of statistical package for social sciences (SPSS version 20). Significant differences were obtained using One Way Analysis of Variance with a probability of 0.05 (95% confidence limit) and Tukeys post hoc test was further used to determine the mean significant differences between specific groups.

Results: Histological findings showed mild, moderate and severe hyperplasia in a dose and time dependant manner. However, observations from quantitative analysisalso revealed a dose and time dependant significant increase in neuronal cell count and cell diameter of the hippocampus, Substantia nigra and cerebellum.

Conclusion: This study has demonstrated that oral exposure of Nicotine in rats display proliferative adaptive changes on the hippocampus, substantia nigra and cerebellum in a dose/time dependent manner.

Keywords: Nicotine, Quantitative analysis, Hippocampus, Substantia nigra and Cerebellum.

Introduction

Cigarette smoking has enormous negative health consequences yet there has been a recorded increase in tobacco consumption.[1] Although 4000 components of nicotine have been described in cigarettes, nicotine is known as the most abundant alkaloid in tobacco.[2,3] Nicotine, a potent parasympathomimetic alkaloid has been shown to accumulate in the roots and leaves of Nightshade family of plants where it constitutes approximately 0.60 – 3.00% of the dry weight of tobacco. This agent ranges between 2- 7 μg/kg of various edible plants consumed through cigarette smoking and tobacco chewing in 30.0 – 40.0% of the world's population. [4] In Nigeria, nicotine is an important constituent of cow urine concoction (CUC) a local remedy used by the Yorubas for the treatment of convulsion in children.[5] Nicotine has been shown to be a natural ingredient in tobacco leaves used as an insecticide in agriculture for pest control.[6]

It has been reported to be the major tobacco alkaloid occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content.[3, 7] Oral snuff and pipe tobacco also has been demonstrated to contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco contributed only about half of the nicotine concentration of the cigarette.[8,9,10] An average tobacco rod contains 10 to 14 mg of nicotine,[11] and on average about 1.00 to 1.50 mg of nicotine is said to be absorbed systemically during smoking.[3]

Nicotine uses and its benefits has been applied in medicine in the treatment of nicotine dependence in order to eliminate smoking. [12] Controlled levels of nicotine were administered to patients in therapy through gums, dermal patches, lozenges, electronic substitute cigarettes and nasal sprays.[12, 13] The relevance of nicotine in health related diseases and as an anti-herbivore chemical has also been reported.[14]

Nicotine from a smoked cigarette has been demonstrated to be circulated to the brain in as little as 7 seconds after inhalation.[15] A typical cigarette contains approximately 0.50 to 1.00 g of tobacco and on average, 10 mg of nicotine,[16] with cigarette smoking of about 10 puffs and within 5 minutes, a typical smoker would have absorbed 1.00 to 2.00 mg of nicotine. Absorption can however, range from 0.5 to 3 mg.[16, 17] The elimination half-life of nicotine is 2 to 3 hours; which implies a reduction in the blood level of nicotine by half after a smoker tries to quit smoking for that particular period.[17]

The addictiveness of nicotine was shown to be as a result of the continual use of tobacco product which resulted in exposure to different carcinogens and other bioactive compounds in tobacco, making tobacco use the leading cause of premature death in developed countries.[18] Addiction to tobacco kills one person prematurely every six seconds.[16] One in two long-term smokers especially in low and middle income countries will die from tobacco addiction.[16]

Matta (2007),[19] classified nicotine as both a stimulant and a depressant based on its ability to cause release of Glucose and epinephrine from the liver and adrenal medulla. As a stimulant, it increases attention, memory, information processing, and learning. [3, 19] Further studieshave suggested that, when smokers desire a stimulating effect, they ingest or inhale short quick puffs, which produces a low level of blood nicotine and generation of action potential.[19]Alternatively, when relaxation was desired, deep puffs are taken which produce a higher level of blood nicotine inhibiting nerve impulse transmission and producing mild sedative effects.[19] At low doses, nicotine potently enhances the actions of norepinephrine and dopamine in the brain, which resulted in drug effect psycho stimulation while at higher doses, nicotine is said to enhance the effect of serotonin and opiate activity, producing a calm, pain-killing effect.[20]It has also been shown to alleviate anxiety, depression, and pain as such, smokers labelled it a stress reliever and were vulnerable to smoke in response to stressful situations or negative moods.[20]

Relationship between cigarette smoking and Parkinson disease was observed by Morens et al. [21] several other investigators have reported a reduced risk of Parkinson disease among smokers and have also provided a strong evidence on the

protective effects of smoking. [22, 23, 24, 25, 26] Nevertheless, biochemical hypothesis of cigarette smoke components, indicated neuroprotection and reduction of enzymatic activity of type B monoamine oxidase in the brain.[27]

Tobacco exposure through the various forms of tobacco consumption has been associated with negative effects on body organ and systems alteration including the brain and nervous system.[23, 26] Studies have suggested that nicotine enhances protective mechanisms on the brain including nerve growth factor deprivation, glutamate induced neurotoxicity and alpha beta mediated cytotoxicity. [23, 26]

Jalili in 2009, carried out a study on morphometrical analysis of the effect of nicotine administration on brain prefrontal region in male rats.[27]Nicotine was administered intraperitoneally for three days; this was to determine the effect of nicotine on neuronal parameters such as neuronal processes, dendritic spine and size of pericarion. The study showed a decrease in the sizes of pericarions and a dose dependant increase in dendritic spines of experimental animals.[27]

Similarly, another experimental and observational study aimed at investigating the effects of prenatal exposure to nicotine on prefrontal cortex of the adolescent rats showed derangement in biochemical profile of the prefrontal cortex in all the experimental groups.[28]According to this study, animals exposed to nicotine during 2nd and 3rd weeks of intrauterine life showed morphological and histological alterations in the prefrontal cortex.[28]

Nicotine has been recognized as a cerebellar toxin.[29] Cerebellar dysfunctions have been linked to lots of neuronal deficits which were believed to be caused by lots of toxins.[29] This could possibly be as a result of the affinity of nicotine agonists to cerebellar nicotine which has been demonstrated from embryonic stage through adult hood as has been documented.[30. 31] Chen et al. in 2003, noted a decrease in the number of purkinje cells in the cerebellar vermis on chronic exposure to nicotine.[32]

A similar experimental study by Johnsen and Miller (1986), on the oral effect of 5.00mg/d and 10.00mg/d of nicotine on male Sprague dawley rats for 60 days reported significant loss of white core of cerebellum.[33] Another similar study also reported that chronic consumption of nicotine exacerbated cerebellar ataxia,[34] and can transiently improve dysartheria, limb and truncal titubation.[31] This was contrary to a study by Lokanadham et al. in 2015,[35]who posited that intraperitoneal administration of nicotine resulted to preservation of the cortical layer and disruption of white core of the cerebellum, Purkinje cell and granular cell layer of the cerebellum. [35] Consequently, a study by Mohammed and his colleagues in 2006, revealed a decrease in purkinje neurons of the cerebellum with an increase in glial fibrillary acidic protein in the white matter and granular layer of the cerebellum.[36]

A cohort study titled exposure of rats to environmental tobacco smoke during cerebellar development alters behaviour and perturbs mitochondrial energetics was carried out by Brian et al in 2012. [37] Rats were exposed to daily environmental tobacco smoke at 300.00 μg/m^3 and 100.00 μg/m^3 from post-natal day 8 to post-natal day 23. The results obtained demonstrated increased locomotor response with significantly perturbed cerebellar mitochondrial subproteome. Also, a dose dependent up regulation of aerobic process was observed. They concluded that the cortical period of cerebellar development was most likely to be affected in environmental tobacco exposure leading to neurobehavioral alterations.[37]

Many pharmacological factors have been known to cause nicotine addiction. The substantia Nigra which has been described as a part of the midbrain dopaminergic systems has received so much concern majorly because of its roles in reinforcement and learning and has been linked to Parkinson's disease.[38] Nicotine was proven to support mesostriatal dopamine antagonists and was also demonstrated to activate the ventral tegmental area and substantia nigra neurons leading to the release of dopamine from the nucleus accumbens.[39, 40]

According to Min et al. (2004), low levels of nicotine comparable with those encountered in smokers not only increased the firing rate of substantia nigra neuron and ventral tegmental dopaminergic neuron invitro but also caused cells to fire more irregularly.[41] They suggested mechanism for this as reported by Min et al. (2004), was that these effects were mediated by activation of nicotinic acetylcholine receptors located both post and pre-synaptically.[41]

Studies have shown physiologically that nicotine levels in the brain enhanced excitatory glutaminergic inputs to ventral tegmental dopamine neurons by activating presynaptic α7 nAchRs.[42] A similar study stated that it was known to modulate inhibitory inputs by activating non α7 nAchRs seen in GABAergic cells, which decreased inhibition of postsynaptic dopaminergic neurons.[43] Chronic administration of nicotine has been reported to facilitate release of dopamine and locomotor responses which has been shown to be as a result of up regulation of dopamine D_2 receptors and led to dopamine turnover.[44] However, a study aimed at analyzing the electrophysiological cause and behavioral consequence of dopaminergic cell loss in knock in mouse strain bearing hypersensitivity nicotinic α4 receptor subunits demonstrated that nicotinic AchRs might be amongst the genes responsible for extensive cell death in substantia nigra than in the ventral tegmental area.[45] The study further hypothesized that nicotine administration induced prolonged excitation of dopaminergic neurons without any morphological change.[45]

Evidence from a study has shown that nicotine improves cognitive function.[46] This theory is related to stimulation of neurotransmitter signals in areas controlling memory and cognitive processes. Their efficacies as putative treatments are highly imperative in impaired cognitive function experienced by patients with Alzheimer's disease.[46] Bergstrom et al. (1996), reported increase in dendritic length of pyramidal neuron and decrease in the size of pyramidal cells in fontal medial cortex following nicotine administration in animals.[47]

Abrous et al. (2002), determined the effect of self-administration of nicotine on hippocampal plasticity.[48] This study involved 180.00μg and 320.00μg doses of nicotine which were administered intravenously. Their finding indicated that nicotine had major effects on the hippocampus where it caused a decrease in PSA – NCAM expression, down regulation of neurogenesis and an increase in cell death by the measure of the number of pyknotic cell degeneration.[48]

Charles in 2011, reported that nicotine was very essential in learning and memory enhancement in experimental animals.[49] He also evaluated its roles in neural cell genesis in the dentate gyrus of the hippocampal formation. Similarly, Charles (2011), also demonstrated the acute and chronic administration of nicotine on the metabolism of amyloid precursor protein (APP).[49] The study revealed that administration of nicotine

influenced the APP metabolism in young rats. The mechanisms which mediated these responses have not been fully elucidated but appeared to depend upon simulation of the nAChR and availability of intracellular free Ca^{2+}.[49] He concluded that the result provided some support for the hypothesis that nicotinic receptor agonists may have some value in slowing the progress of Alzheimer's disease.[49]

A cohort study aimed at investigating some of the effects of both ethanolic and smoke tobacco on the hippocampus in which tobacco extracts were administered via oral and inhalational route at 10.72µg, revealed more population of cells in experimental animals compared to control.[50] They further concluded that consumption of nicotine via either smoking or chewing tobacco may lead to some level of neurohistoarchitectural alterations, brain weight changes and neurobehavioural disruption.[50]

Alim et al. (2012), reported that short term administration of low dose nicotine enhanced memory processes and improved the oxidative stress status of the brain. [51] They however stressed that nicotine may have contributed to the neuroprotective effects of tobacco use in Parkinson disease.[51]

A model of nicotinic replacement therapy during pregnancy and breastfeeding were used to assess the consequences of chronic exposure of nicotine in cerebral neuroplasticity in offspring.[52] Dawley rats exposed to nicotine throughout prenatal and postnatal development displayed no significant alteration in dentate gyrus neurogenesis. Contrary to this findings were that long term potentiation of nicotine led to a significant increase in dentate gyrus.[52]

A study on the hippocampal and striatal histomorphology following chronic nicotine administration in male and female rats was carried out by Ijomone et al. in 2015. [53] Nicotine was administered at a dose of 0.25, 2.00 and 4.00µg/kg for 28 days. This study showed a significant increase in percentage of neurons with degenerative features in hippocampus and striatum of both male and female rats.[54]

Studies have x-rayed in details the deleterious effects of smoking and nicotine on the brain, liver, lungs, kidney, ovary and testicular histology.[54, 55, 56, 57] Scanty literatures have been specifically devoted to the evaluation of nicotine on histology and quantitative analysis of the Cerebellum, Hippocampus and substantia Nigra.[56, 58] Hence this research was aimed at evaluating the impact of orally ingested nicotine on the histology and cell count analysis of the Cerebellum, Hippocampus and substantia Nigra. These selected parts of the brain have been demonstrated to contain different forms of nicotinic receptors, forms a major part of the mesolimbic system and are directly or indirectly involved in the control of movement.

Materials and Methods

Animal and diet

Adult male Wistar rats with an average body weight of 100g – 200g were purchased from animal house of Delta State University Abraka. All animals were fed with commercially formulated rat chowand water.

Chemical compound

The experimental drug was nicotine (*(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine*).25.0g of nicotine hydrogen salt tartrate [(95% nicotine); Sigma life science, 614-002-00-x United Kingdom (batch number: USAB313 0016, Expiry date: November, 2020)] was purchased from Rovet Scientific shop in Benin-City, Edo State, Nigeria.

Ethical consideration

Approval for this study was obtained from the Faculty of Basic Medical Science ethical committee Delta State University Abraka. This conforms to the guidelines for animal researches stated by Animal research ethics in 2009.[59]

Table 1: Experimental groups and Nicotine administration

Groups	Designation	Duration and Treatments		
		Sub-acute (7 days)	Acute (21 days)	Chronic (42 days)
1	Control	Distilled water		
2	Low dose	2mg/kg body weight of nicotine		
3	Moderate dose	4mg/kg body weight of nicotine		
4	Chronic dose	6mg/kg body weight of nicotine		

Sample size

This was determined using the resource equation E = Total number of animals " Total number of groups. [60. 61]

Where E is the degree of freedom for the analysis of variance.[60]

A total number of 24 male adult Wistar rats were used for this study.

Study design

This was an experimental and observational study that entailed four groups (1 - 4). The rats were weighed, sorted and assigned to a group containing four rats each. Animals were fed with commercially formulated rat chow and water ad libithium under standard condition (12 hours light and 12 hours darkness, temp: 28-31^{0}C; humidity: 50-55%) and were allowed to acclimate for two days before administration according to animal acclimatization guidelines stated by Obernier and Baldwin (2008); Animal research ethics(2009).[59, 62] The experiment lasted for 6 weeks with animals euthanized at day 7, 21 and day 42; this was to demonstrate the time and dose dependent effect of the agent.

Dosage and drug administration

Diluent for the administration of nicotine used was water and time of administration was twice daily (6.00 am and 6.00pm); this was to maintain a steady concentration of nicotine in blood circulation Route of administration of test drug was oral with the use of orogastric tube and experimental animals were treated as indicated in (table 1).

The LD50 for oral nicotine administration in adult male rats is 50mg. [3]

Inclusion and Exclusion criteria.

The same species of rats and rats with similar body size were used for this study. Rats with open wounds, other signs of illness or injury and rats with physical signs of oedema were excluded from this research.[63]

Animal euthanasia.

Experimental rats were euthanized at 7, 21 day and 42 day by cervical dislocation after an overnight fast. [64] Brain tissues were harvested and fixed in bouin's fluid.[65]

Histological procedures

Tissues from the substantia nigra, hippocampus and cerebellum were processed and stained using standard histological technique.[65]

Stereological analysis

Neuronal cell counts and cell diameters were determined by taking the mean of cells sampled in a grid, using a Microscope at a magnification of x100 which had an image analyzer powered to a computer.[66]

Statistical analysis

Results of cells dimension were expressed as mean ± SEM. Data were subjected to SPSS (Version 20), and were analyzed using one way ANOVA at a confidence level of 95.0% which was used to test differences between the means of the parameters in the experimental groups. Levels of significance were determined at ($P < 0.05$) and Tukey Post Hoc test was further used to determine the significant differences between the means of specific groups.

Photomicrography

Haematoxylin and Eosin Stained tissue images were captured using digital microscopic eyepiece "SCOPETEK" DCM 500, 5.0 mega pixel connected to computer

Table 2: Histomorphologic effects of nicotine on cerebellar cortex neuronal count from animals sacrificed on day seven (7)

Doses administered	Molecular layer(μm)	Purkinje layer(μm)	Granular Layer(μm)
0mg/kg body weight	4.28±0.43[a]	1.45±0.15[a]	43.81±4.56[a]
2mg/kg body weight	6.18±0.51[b]	2.00±0.26[a]	53.08±5.99[b]
4mg/kg body weight	8.12±0.77[b]	2.35±0.29[a]	64.36±5.91[a]
6mg/kg b0dy weight	8.51±0.65[b]	2.89±0.39[a]	80.89±5.81[b]

Values are expressed as Mean ± SEM for n = 4 rats.Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Table 3: Histomorphologic effects of nicotine on cerebellar cortex neuronal count from animals sacrificed on day twenty one (21)

Doses administered	Molecular layer(μm)	Purkinje layer(μm)	Granular layer(μm)
0mg/kg body weight	5.36±0.35[a]	2.00±0.37[a]	57.47±4.78[a]
2mg/kg body weight	10.91±1.21[b]	2.31±0.32[b]	78.10±5.89[b]
4mg/kg body weight	7.66±0.84[b]	2.06±0.21[a]	72.63±4.49[a]
6mg/kg b0dy weight	9.16±0.82[b]	2.35±0.32[b]	78.50±4.05[b]

Values are expressed as Mean ± SEM for n = 4 rats.Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Results

Figure 1 showed the cerebellar cortex made up of molecular layer, purkinje layer and granular layer. The molecular layer (ML) was composed of neurons with basophilic nuclei peripherally located. The Purkinje cells in the Purkinje layer (PL) were seen with a granulated eosinophilic cytoplasm and a lightly stained round to oval granulated centrally placed nuclei. Abundant neuronal nuclei disposed in clusters and granulated in appearance were seen in the granular layer (GL). The cell membrane appeared indistinct. Features revealed mild, moderate and severe hyperplasia of the cerebellar cortex across group with increase in dose and duration of exposure

Figure 2 showed the Substantia nigra cytoarchitectural features of experimental animals made up of numerous pigmented multipolar neuronal cell bodies, axons and dendrites. The pigmented neuronal cytoplasm was eosinophilic. The nuclei were granulated, round to oval in shape with a non-prominent nucleoli peripherally placed embedded in an indistinct cell membrane. Also seen were abundant oligodendrocytes with a centrally placed nuclei and pericytoplasmic hallow. These features displayed mild, moderate and severe hyperplasia in a dose and time dependant manner.

Histological sections in figure 3 showed the layers of cornus ammonis (CA) and dentate gyrus (DG). The polymorphic layer (PL) was made up of few neurons. Next to this layer was the thick granular layer (GL) which was made up of abundant granular cells with deeply basophilic nuclei centrally located occupying almost the whole cytoplasm. The stratum oriens was made up abundant of pyramidal cell fibres (SO). Next to the stratum oriens was the stratum pyramidale layer (SP) which was composed of abundant pyramidal cell. The pyramidal cells had triangular shaped cell bodies with large, round to oval nucleus centrally placed. The nuclear cytoplasmic ratio was 3:4 and the nucleoplasm was composed of dense chromatin granules. Also seen were abundant oligodendrocytes with centrally placed nucleus and pericytoplasmic hallow. Features indicated mild, moderate and severe hyperplasia of the hippocampus with Gliosis.

Stereology result in table 2 above showed a significant (P=0.00) increase in the cell count of the molecular, purkinje and granular neurons across the group. Following the cell count, severe hyperplasia was prevalent in group four (4) which received the highest dose (6mg/kg body weight) of the test drug. This notable change in the quantitative analysis in the fourth group was significant (P =0.00) and was time and dosage dependent

From table 3 above, rats administered 2mg/kg body weight of nicotine showed a significant transient (P=0.000) increase in both molecular and granular neurons with a non-significant (P = 0.809) increase in the Purkinje layer on exposure to nicotine. Test animals administered 4mg/kg body weight of nicotine showed a non-significant increase in the cell count in all the three layers. However, similar significant (P<0.001) changes between rats administered 2mg/kg body weight of nicotine and rats administered 6mg/kg body weight of nicotine were also noted with a slight increase from 7.66±0.84 in group three (3) to 9.16±0.82 in molecular layer of the group that was administered 6mg/kg body weight of nicotine test animals.

Results from table 4 above indicated hyperplasia of the three layers of the cerebellar cell count on administration of nicotine. Subsequent administration of nicotine with increased time and dosage showed a significant (P = 0.000) increase in the test groups cerebellar cortex as compared to the control group. Severe hyperplasia was evident in the group that was administered 4mg/kg body weight of nicotine which received highest dose of the test drug and caused a significant (P<0.000) change in the neurons across the group. An exception to this is in the Purkinje layer of group 3 rats that was administered 4mg/kg body weight of nicotine showed a no significant increase in the Purkinje neurons on exposure to nicotine (P>0.05).

The experimental groups all showed significant increase (P=0.00) in the quantitative analysis of the histology of the substantia nigra when compared to control with increased duration and dosage across the groups. Maximum significant increase (P=0.00) were noted in group two test animals (2mg/kg body weight) of day twenty one (21), group four test animals (6mg/kg body weight) of day twenty one (21) and day forty two (42).

Result from table 6 showed that the mean cell count of the granular layer increased rapidly in the experimental rats treated with nicotine for seven (7) and twenty one (21) days. This statistical significant (p=0.002) changes is however evident in groups 2 (2mg/kg body weight), 3 (4mg/kg body weight) and 4 (6mg/kg body weight) in 7 days and group 2 (2mg/kg body weight) and 4 (6mg/kg body weight) in 21 days. Animals treated for 42 days also showed increases in granular cells which was not significant in all the groups (P>0.00).

The result in table 7 above shows a significant (P=0.00)

Table 4: Histomorphologic effects of nicotine on cerebellar cortex neuronal count from animals sacrificed on day forty two (42)

Doses administered	Molecular layer(μm)	Purkinje layer(μm)	Granular layer(μm)
0mg/kg body weight	5.43±0.35[a]	1.75±0.19[a]	56.46±4.58[a]
2mg/kg body weight	8.30±0.63[b]	2.30±0.26[b]	61.51±4.84[b]
4mg/kg body weight	7.57±0.82[b]	2.87±0.31[a]	67.50±2.34[b]
6mg/kg body weight	14.1±1.5b	4.00±0.67[b]	117.14±7.47[b]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

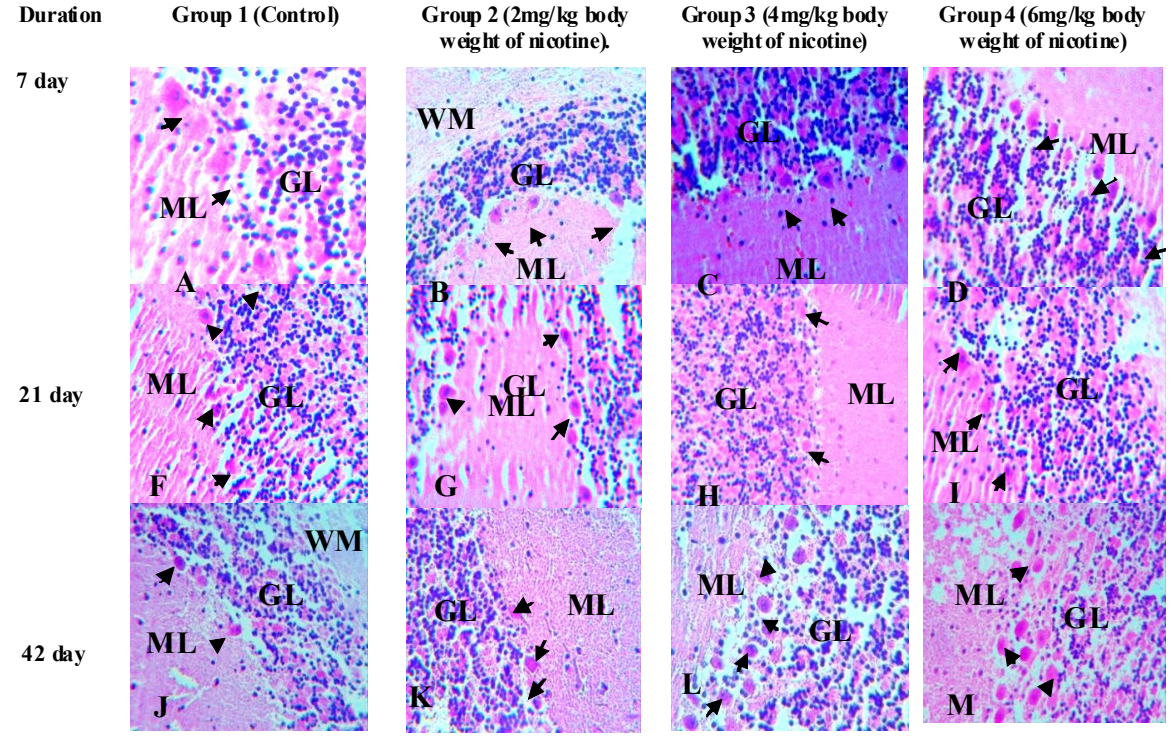

Figure 1: Histological effects of Nicotine on the cerebellum of control and experimental rats exposed to different doses of nicotine for 7, 21 and 42 days.
H and E (x400)
Keywords: WM: White mater, arrow head (➹): Purkinje cells; ML: Molecular layer; GL: Granular layer

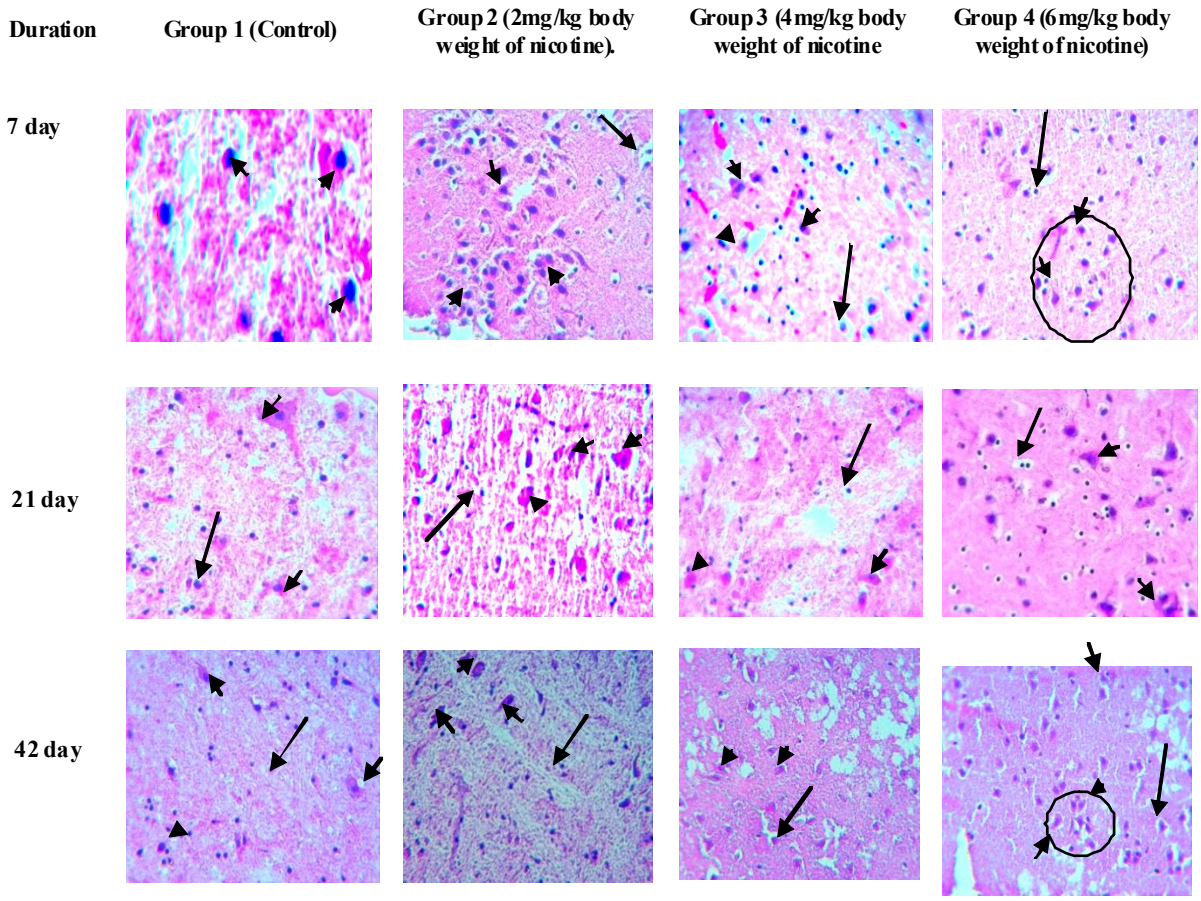

Figure 2: Histological effects of Nicotine on the Substantia nigra of control and experimental rats exposed to different doses of nicotine for 7, 21 and 42 days.
H and E (x400).
Keywords; Arrow head: pigmented neurons of the Substantia nigra; Arrow: Oligodendrocytes.

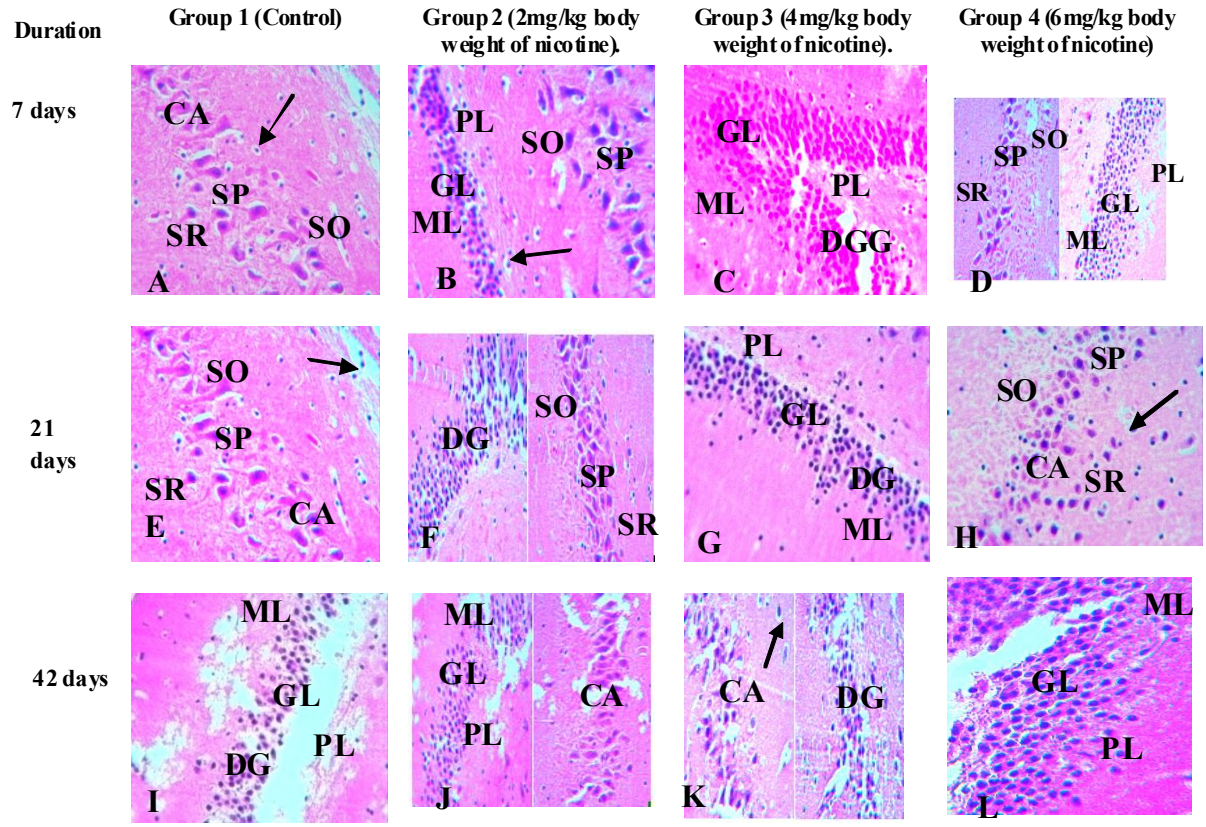

Figure 3: Histological effects of Nicotine on the Cornus ammonis and Dentate gyrus of control and experimental rats exposed to different doses of nicotine for 7, 21 and 42 days.
H and E (x400).
Keywords; DG: Dentate gyrus, CA: Cornus ammonis, SO: Stratum oriens, SP: Stratum pyramidale, SR: Stratum radiatum, ML: Molecular layer, GL: Granular layer, PL: Polymorphic layer, Arrow: Oligodendrocyte

increase in the cell count of the pyramidal neurons across the group. Following the cell count, severe hyperplasia was prevalent in group four (6mg/kg body weight) with increase duration of nicotine. This notable change in the quantitative analysis in the fourth group that received 6mg/kg body weight of nicotine is significant (P=0.00) and is seen in group 2 (2mg/kg body weight), 3 (4mg/kg body weight) and 4 (6mg/kg body weight) in day seven and twenty one. However, a similar increase which was (P=0.05) also seen in group 3 (4mg/kg body weight) animals exposed to test drug for a period of 42 days.

Table 8 shows that the maximum diameter of the dopamine producing neuron of the Substantia nigra is seen in group four (6mg/kg body weight) treated for 21 days. Cell count increases were apparent between day seven and day twenty one treated animals which is not significant. Comparing neuronal diameter of day forty two animals to the twenty one test animals showed a slight significant decrease in test animals administered nicotine for forty two days. Statistically significant groups (p= 0.02) were seen in group 3 (4mg/kg body weight) and group 4 (6mg/kg body weight) test animals sacrificed on day forty two.

The result in table 9 above shows a significant (P=0.002) increase in the diameter of the Purkinje neurons across the group. Following the cell count, severe hyperplasia was prevalent in group four (6mg/kg body weight) with increase duration of nicotine. Maximum significant diameter is seen in group 4 (6mg/kg body weight) of animals exposed to nicotine for twenty one days, with a very slight significant decline between this group and group four (6mg/kg body weight) of animals treated for forty two days.

Results in table 10 showed animals exposed to test drugs for seven days showed a statistical significant increase in pyramidal cell diameter across the group. With subsequent administration of nicotine for twenty one and forty two days, there was a non-significant (P= 0.068) increase in pyramidal cell diameter

Table 5: Histomorphologic effects of nicotine on Substantia Nigra dopamine producing neuronal count from animals sacrificed on day 7, 21 and 42

Doses administered	Day seven(µm)	Day twenty one(µm)	Day forty two(µm)
0mg/kg body weight	3.29±0.31[a]	2.00±0.16[a]	3.48±0.20[a]
2mg/kg body weight	5.58±0.64[b]	3.83±0.25[b]	4.02±0.22[b]
4mg/kg body weight	6.55±0.32[b]	7.12±0.44[b]	6.14±0.22[b]
6mg/kg body weight	7.12±0.37[b]	8.94±0.51[b]	8.90±0.42[b]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Table 6: Histomorphologic effects of nicotine granular neuronal count of the dentate gyrus from animals sacrificed on day seven, day twenty one and forty two

Doses administered	Day seven(µm)	Day twenty one(µm)	Day forty two(µm)
0mg/kg body weight	18.44±1.96[a]	29.44±3.74[a]	35.29±2.47[a]
2mg/kg body weight	20.21±2.32[b]	38.38±3.22[b]	37.5±3.56[a]
4mg/kg body weight	48.00±5.79[b]	47.47±4.18[a]	42.27±2.82[a]
6mg/kg body weight	51.42±7.35[b]	58.00±5.85[b]	46.60±2.97[a]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Table 7: Histomorphologic effects of nicotine pyramidal neuronal count of the cornus ammonis from animals sacrificed on day 7, day twenty one and forty two

Doses administered	Day seven(μm)	Day twenty one(μm)	Day forty two(μm)
0mg/kg body weight	3.38±0.60[a]	8.09±0.96[a]	12.00±0.86[a]
2mg/kg body weight	6.58±0.92[b]	12.63±1.19[a]	15.83±1.89[a]
4mg/kg body weight	8.85±1.27[b]	14.00±1.39[a]	16.54±1.13[b]
6mg/kg body weight	11.90±3.57[b]	18.92±1.84[a]	22.61±1.81[b]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work).

Table 8: Effect of nicotine on dopamine producing neuron diameter from animals sacrificed on day 7, day twenty one and forty two

Doses administered	Day seven(μm)	Day twenty one(μm)	Day forty two(μm)
0mg/kg body weight	34.85±1.77[a]	88.72±7.86[a]	53.79±2.09[a]
2mg/kg body weight	41.02±6.11[a]	94.39±7.72[a]	76.18±9.47[a]
4mg/kg body weight	55.95±11.82[a]	86.47±1.77[a]	82.07±4.18[b]
6mg/kg body weight	59.73±2.33[a]	103.14±7.19[a]	84.70±2.50[b]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Table 9: Effect of nicotine on purkinje cells diameter from animals sacrificed on day 7, day twenty one and forty two

Doses administered	Day seven (μm)	Day twenty one (μm)	Day forty two(μm)
0mg/kg body weight	55.50±2.25[a]	62.00±4.51[a]	49.39±3.04[a]
2mg/kg body weight	55.70±2.02[b]	78.00±1.52[a]	61.86±4.47[a]
4mg/kg body weight	66.00±2.30[a]	81.90±0.58[a]	70.87±2.23[a]
6mg/kg body weight	72.66±6.35[b]	87.25±7.20[a]	87.07±6.48[a]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

Table 10: Effect of nicotine on pyramidal cells diameter from animals sacrificed on day 7, day twenty one and forty two

Doses administered	Day seven	Day twenty one	Day forty two
0mg/kg body weight	70.85±0.88[a]	64.69±3.99[a]	76.32±5.60[a]
2mg/kg body weight	75.4±0.78[b]	88.84±10.05[a]	88.05±3.00[a]
4mg/kg body weight	84.93±1.88[a]	80.53±2.40[a]	93.67±9.66[a]
6mg/kg body weight	95.22±6.27[b]	85.78±1.79[a]	102.46±0.63[a]

Values are expressed as Mean ± SEM for n = 4 rats. Values that bear another superscript on a column differ significantly (P<0.05). (Source; Field work)

between animals administered nicotine for twenty one days and forty days respectively.

Discussion

The present study examined the histomorphological, varying dose/time effects of nicotine in the hippocampus, cerebellum and substantia Nigra. Several studies on neuronal interactions of nicotine have highlighted the adverse and beneficial potentials of this agent on the brain and thus, have raised many controversies. [22, 23, 24, 25] However, judging from the scientific investigations conducted on the clinical significance of nicotine, it has become paradoxical to note that studies that employed the use of histological, stereological and statistical tools in exploring the neuronal responses to these agents have been few while available ones did not employ the use of statistical tools as a basis for their arguments.[67]

This index study clearly demonstrated hyperplasia in cell population and hypertrophy. Hypertrophy has been described as physiological response to a specific stimulus that results to an increase in the amount of organic tissue that results from proliferation. [68] Hyperplasia has been described as an adaptive response in replicating cells and in cells induced to replicate by an introduction of an agent. Hypertrophy and hyperplasia may occur independently but in some cases, they can occur together as demonstrated from findings of this study.[68]

Histological changes in the cerebellum.

Cerebellar areas have been reported to be imperative in integration and regulation of well-coordinated muscular activities, regulation of tone, posture and equilibrium by regulating impulses from tactile, proprioception, visual and auditory receptors. [69] The cerebellar hemisphere is the major channel that connects sensory areas of the brain to motor areas of the brain. It forms a relationship during coordinated movement which is involved in motor learning and modifications of reflexes.[31] The cerebellum has been reported to be susceptible to toxic chemical compounds and drugs at higher doses; granular layer and Purkinje neurons are usually more vulnerable compared to the molecular layer which is least affected.[70]Amongst the cerebellar toxins as described by Manto, [70] low dose of nicotine was shown to be neuro-protective in the cerebellum. [27]

The current study demonstrated that oral administration of nicotine for seven days resulted in mild hyperplasia compared to experimental rats exposed to nicotine for 21 and 42 days. Findings from the molecular layer of the cerebellum showed preserved histoarchitetural and cytoarchitectural features of a viable neuron with increased proliferation of neuronal cells. These however, suggested a proliferative effect of nicotine and could be as a result of the low dosage and short duration of exposure to nicotine but disagrees with findings by Lokanadham et al, who reported neuronal degeneration in the cerebellum.[35] Reasons for the dissimilarity could be the longer duration and higher dosage of nicotine administration to test animals. Further studies should employ the use of histochemical stains such as cresyl violet to demonstrate the viability of neurons in the cerebellar layers.

Purkinje neurons exhibited well preserved and non-distorted neuronal characteristics with a transient increase in the Purkinje cell number which was suggestive of mild hyperplasia. This finding is contrary to a study conducted by Omtosho and Babalola (2014), who observed a degree of shrinkage in animals exposed to cigarette smoke.[28] Differences in observation could be that cigarette smoke has been demonstrated to contain several active components which have been demonstrated to work synergistically to produce neurodegeneration of Purkinje cells previously documented. [3, 28] However, for further studies, phytochemical screening of cigarettes and tobacco should be employed; so as to separate the active ingredients in cigarette and the individual components should be tested on experimental animals to indicate the specific components that produce the observable effect.

The granular layer of the cerebellum on exposure to nicotine for seven days showed brain parenchyma preservation, and increase in cellularity. The increase in cellularity of the granular layer neurons is suggestive of mild hyperplasia as compared to other groups. These findings disagrees with a study conducted by Lokanadham et al. in 2015,[35] who concluded that long term

exposure of nicotine results to loss in cellularity of the granular layer. Dissimilarity in the finding with that of the current study could be as a result of the route of nicotine administration. The latter study administered nicotine intraperitoneally which is similar to intravenous route and releases a large concentration of nicotine to blood concentration as compared to oral route which is extensively metabolized in the liver and cleared in the kidney.[71, 72] Other routes of drug administration can also be employed so as to compare the various effects produced on nicotine administration.

The molecular layers of the animals administered nicotine for 21 days, showed increase in cellularity as compared to experimental animals administered nicotine for 7 days with absence of morphological alterations. This neuronal proliferation could be attributed to the neuro-protective effect of nicotine, and the small dosage of nicotine administration. This agrees with the inference that low doses of nicotine poses a stimulating effect on the central nervous system.[3, 19, 26] The reason for this is likely due to the fact that the molecular layer is least susceptible to chemical toxins and drugs. [70]

On administration of nicotine for twenty one days, the purkinje cells showed increase in cellularity suggestive of moderate hyperplasia as compared to other groups. This Purkinje cells histoarchitectural and cytoarchitectural cellularity is contrary to a study which demonstrated disruption of the Purkinje cell. [35] Reasons for this dissimilarity between the present study and that of Lokanadham and his colleagues could be ascribed to intraperitoneal route of nicotine administration and difference in nicotine dosage employed in the two studies. However, a future study should involve another means of drug administration such as inhalational route which has been reported as the fastest route for nicotine administration.[73]

The deep granular cell layer of the cortex maintained its histological characteristics but with a dose dependant increase in the granular neuron which also suggests moderate hyperplasia without morphologic disruption. This observation does not concur with findings from a study by Lokanadham et al. (2015), who discovered granular cell disruption that could invariably lead to cerebellar disorders such as cerebellar ataxia that can transiently improve dysartheria as reported by Tewari (2010) and Houk *et al.* (1993). [31, 34] Possible reasons for the observed differences could be attributed to differences in nicotine metabolism and renal clearance between the two different routes of administration in the study; but however, further studies should be carried out on the toxicological analysis of nicotine to buttress this finding.

A dose dependant severe hyperplastic change was seen in the cerebellar molecular layer of rats exposed to nicotine for the long duration of exposure. This finding is in agreement with the finding that chronic administration of nicotine for 42 days maintains the cerebellar histological characteristics and does not induce neuronal impairment as previously reported.[29]

The Purkinje cells play an important role in development and maturation of the cerebellum.[74] It has been reported to exert an inductive effect on the development of the cerebellar granule layer; and also is the most likely vulnerable layer susceptible to toxins.[70, 74] The Purkinje cell showed no neurodegeneration with an increase in duration of exposure but rather a marked and dose dependant increase in cellularity which suggests severe hyperplasia as compared to other groups. This finding is contrary to a study conducted by Mohammed et al. (2006), who reported Purkinje cell degeneration on chronic and long term exposure to nicotine. [36] The reason for this might be attributed to the in-utero exposure of nicotine during the embryonic period of development in Mohammed's study. The embryonic period of development has often been described as the most critical and sensitive time of induction of birth defects.[75] Further studies should however consider administration of nicotine in-utero but during the fetal period; as it is a period with reduced risk of gross structural abnormalities

Granular cells accounts majorly for most of the neurons in the human brain and hence, play a vital role in determination of Cerebellar functions.[76] The deep granular layer on exposure to nicotine indicated a similar resultant pattern as in day seven and day twenty one but with severe hyperplasia. This observation could be explained by the mechanism of action of nicotine or its antioxidant effects as previously reported by Imosemi, *et al.* in 2013.[77] Receptors in the granular layer includes glutamate receptors, nicotinic and muscarinic receptors; nicotine has been proven to enhance excitatory glutaminergic inputs by activating presynaptic ±7nAchRs. [42, 78] The present study did not provide a molecular basis for this observation and may be considered in future investigations.

Quantitative changes in the cerebellum.

The present study demonstrated a dose dependant significant increase ($P < 0.05$) in neuronal count of the molecular layer of rats exposed to nicotine for seven days. This finding has been related to the fact that the molecular layer is the least of the cerebellar layers to be affected by chemical compounds as noted by Fonum and lock in 2000.[74] This hypothetical analysis did not concur with the report of Tewari in 2010 who explained molecular hypoplasia following long term administration of nicotine.[31]

Purkinje cells morphology have been frequently reported using both quantitative and qualitative methods.[79, 80] Purkinje cell count revealed a significant increase in Purkinje cells especially in the group that received the highest dose of nicotine for seven days. Result from Purkinje cell diameter further showed a dose dependant significant increase in the diameter of the cell membrane. These observations strongly provided evidence on the hyperplastic and hypertrophic activity of nicotine and do not agree with findings by Chen et al, (2003),[32] who reported decrease in Purkinje cells. Possible reasons for the result differences could be the fact that Chen did not employ any stereological technique and rather based his findings on observations which did not provide any statistical basis for hypothetical testing. For further studies, the Cavalieri's principle should be used in the cell count because it expresses volume number connectivity and the linear biological structures are expressed as absolute values.[81]

Quantitative analysis of the granular layer revealed a dose dependant significant increase in granular layer cell count; however, Omotosho and Babalola (2014),[67] reported reduction of granular cell number. Reasons for this disparity account to the different route of drug administration used by Omotosho and Babalola who also based his quantitative analysis on mere observations without the use of stereological grid counting techniques and hence his findings lacked hypothetical deductions. They results from the quantitative analysis therefore implied that nicotine exerted hyperplasic and hypertrophic effect on the cerebellum

The molecular layer showed an increase in cell count, which

was coupled with a simultaneous increase in the cell layers. This significant increase was very apparent in group 2 and group 4 and is suggestive of mild to moderate hyperplasia of this layer; however, the study by Tewari and his colleagues in 2010,[31] reported a significant loss in central core and molecular layer of the cerebellum. The existing controversies in this finding could be a result of an aluminium foil which was covered with the nicotine containing cannula used in the study by Tewari et al. 2010. Aluminium has been demonstrated to be a powerful toxin in nervous system embryology.[82] Similar observations were noted on exposure of nicotine to experimental animals, a dose dependant increase ($P > 0.05$) in Purkinje cell proliferation and a significant dose dependent increase in Purkinje cell diameter was recorded. The findings in the purkinje layer could be related to the neuro-behavioral state of the animals such as depression which has been reported to affect growth of neurons.[83] However, for further studies nicotine administration should be examined together with neurobehavioral studies so as to correlate the observable differences.

The administration of nicotine to experimental rats increased the number of neuronal cells of the granular layer. This increase suggests a moderate hyperplastic effect of nicotine. Moderate hyperplasia was present with an average number of cells in viable neurons present within this layer. This result could be as a result of the mechanism of action of nicotine on the nAChRs in the granular layer.

With increased duration and exposure of nicotine, the molecular layer showed a dose dependant significant severe hyperplasia down the group ($P < 0.05^2$). This finding could be as a result of the possible positive interactions between the nAChRs in the brain and nicotinic agonist such as nicotine.

The extensive severe hyperplasia of Purkinje cell was very evident and significant with increase in dosage and exposure of experimental animals to nicotine. Purkinje neurons which have been reported to be very vulnerable to chemical compounds in the brain also showed marked and dose dependent increase in Purkinje cell diameter. This observation further hypothesizes the moderate hyperplastic and hypertrophic effect of nicotine.

Quantitative analysis of the granular neurons showed a dose dependant significant increase in cell count. This increase was very significant across and within the groups. It further infers that nicotine agonists (nicotine) exert a severe hyperplastic effect on the granular neurons. This observable increased cellularity could be explained with the longer period of nicotine exposure to model animals and has not been previously reported.

The cerebellar circuit is made up of climbing fibers, mossy fibres and parallel fibres and they are all excitatory and may probably use L- glutamate as neurotransmitter.[78] Purkinje cells have been shown to be made up of GABA while receptors in granular layer mediating glutaminergic and cholinergic transmissions include ionotropic (glutamate receptors of NMDA (N- Methyl – D- aspartate) and Non NMDA type A metabotropic (coupled to 2nd messengers systems), Glutamate receptors, Nicotinic and muscarinic acetylcholine receptors.[78] Mansvelder (2002),[42] reported that nicotine is known to modulate inhibitory inputs by activating non ±7 nAchRs seen in GABAergic cells, which decreases inhibition to postsynaptic neurons. This might be a likely mechanism for the neuroprotective effect exerted by nicotine in the cerebellum.

Histological Changes in the Substantia Nigra.

The substantia nigra is highly essential in the control of fine motor movement.[84] Many of its cells have been shown to contain neuromelanin and its cells give rise to nigrostriatal fibres which are dopaminergic and appear to act as a neurotransmitter causing inhibitory effects particularly on neurons in the corpus striatum.[84] Loss of about 80% of its dopamininergic cells is a fundamental defect in Parkinsonism.[84]

This study showed that experimental animals, administered nicotine for seven days were presented with no morphological alterations and neurodegeneration and mild hyperplastic changes were observed in the tissue architecture which indicated that with administration of nicotine, there was a dose dependant increase in cellularity in all the groups treated with nicotine. Mild hyperplasia has been shown to be a neuronal physiologic response characterized by little or few increase in cell number.[68] This observable change in the substantia nigra could be linked to the neurogenesis potentials of nicotine reported by Eriksson *et al.* in 1998.[85] Neuronal growth and generations from progenitor cells have also been demonstrated in rats' substantia nigra.[86] However, further studies should investigate the neurogenesis potentials on areas of the brain using biomarkers or antigens specific for nerve growth factor which is responsible for neuron generation.

In the current study, mild to moderate hyperplasia was observed in substantia nigra of animals exposed to nicotine for twenty one days. These mild to moderate proliferation of pigmented neurons increased as treatment continued with varying doses. The resultant pattern revealed the proliferative effect of nicotine. This activity of nicotine was further buttressed with the fact that nicotine induced development of new neuronal cell line and thus suggests neurogenesis potentials observed in animals treated for twenty one days. This could be linked to the stimulating effect of nicotine; where nicotine, acted on neurons and improved cellularity in certain regions of the brains.[3, 19]

The present study indicated that nicotine exerted a severe hyperplastic effect on pigmented neuron of the substantia nigra. Nicotine also conferred an improvement of cellularity and architectural features in the experimental groups and may possibly be as a result of nicotine interactions on the dopamine receptors seen in the substantia nigra. It has been shown that chronic administration of nicotine facilitates release of dopamine and locomotor responses in rats.[45] A previous study reported that nicotine acts on dopaminegic D1 and D2 receptors which invariably leads to up regulation of the dopamine receptors, a condition described as dopamine turnover.[45]

Quantitative changes in Substantia Nigra

Stereological techniques have often been used in describing and quantifying neuronal count.[87] Terms such as many, few, numerous are inappropriate in describing hypoplastic and hyperplastic tissue changes but provides basis for hypothetical testing in neuronal quantification.[88]

Pigmented neuron showed a dose dependant significant increase in cell count as compared to the control group. This significant increase was very transient and rapid in the group that received the highest dose for seven days. This observation confirmed the mild hyperplasic effect exerted by nicotine in substantia nigra histology. The cell diameter showed a similar increase ($P > 0.05$) which also indicated increase pigmentation in the neuron. These findings therefore, could be linked to the stimulating effect of dopamine by nicotine as previously reported.[3, 19] Reasons for a no significant finding could be that a fraction of the drug which remained unchanged after metabolism

and renal clearance was not potent enough to cause a significant effect.

The current study evaluated the dose dependent increase in neuronal count of the pigmented neurons exposed to nicotine for twenty one days which revealed significant increase in pigmented cell number across the groups. This finding has critically explained the reasons for the observed increase in cellularity as documented in the histology and further reinforces the hypothesis that nicotine exerted a significant moderate hyperplastic effect on the substantia nigra. On cell dimensions, neuron thickness revealed a physiologic hypertrophy which was also not significant. Possible reasons for this could be similar to that observed in day seven experimental animals and could be attributed to the synaptogenic effect of nicotine as has particularly been reported, where it facilitated formation of synapse and action potential by opening of calcium channels.[89, 90] For further studies, studies should evaluate brain nicotine receptor response on administration of nicotine using electroencephalogram.

Experimental animals administered nicotine for forty two days showed extensive severe hyperplasia which corresponded to the severe hyperplastic changes documented in the histology of substantia nigra in day 42 rats. Severe hypertrophy was also demonstrated in the cell diameter which was caused by increased nicotinic receptor surge in the cell thickness and also an increase in neuromelanin pigmentation in substantia nigra neurons. This finding therefore ascertained the fact that chronic administration of nicotine induced stimulation and production of dopamine pigmentation which caused them to fire rapidly as documented by Min et al. in 2004.[41] This notable change could be ascribed to the fact that neuromelanin which causes pigmentation in the substantia nigra neurons has been reported to sequester chemicals.[91] More so, these findings concurs with that of Sabine et al. (2004),[45] who observed no morphological changes on but rather an induced prolonged excitation of dopaminergic neurons on nicotine administration

Likely mechanism of action of nicotine on substantia nigra is that; the substantia nigra pars compacta is made up of dopaminergic and cholinergic neurons which contain neuromelanin pigment granules while the ventral pars recticularis contains fewer neurons grouped in clusters some of which are dopaminergic while a major of them are gabaergic.[78] Nicotine agonist has been shown to interact with these receptors as noted by Min and his colleagues,who demonstrated that nicotine effects are mediated by activation of nAChRs located both postsynaptically and presynaptically. [44] A cohort Study also demonstrated physiologically that nicotine levels in the brain enhanced excitatory glutaminergic inputs to ventral tegmental dopamine neurons by activating presynaptic α7 nAchRs.[42] A similar study also explained that it was known to modulate inhibitory inputs by activating non ±7 nAchRs seen in GABAergic cells, which decreases inhibition to postsynaptic dopaminergic neurons.[43]

Nicotine has been related in the management of parkinsonism which has been reported to be seen in conditions that share damage to dopaminergic neurons [68]. It is a clinical movement disorder characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait and tremor.[68] Damage to about 80% of dopaminergic neuron is a defect in parkinsonism.[85] The present study showed no percentage of neuronal damage on administration of nicotine and therefore implies that nicotine can be used in the management of Parkinson disease (PD). A study showed patients who smoke, are 50% less likely to have PD when compared to their non-smoking counterparts.[92] This however, suggests that nicotine a content of cigarette and tobacco might exert a neuroprotective effect against degeneration and thereby preventing PD. This observation concurs with similar studies which stated nicotine simulates dopamine release and thereby suppresses early signs of PD. [92, 93]

Histological changes in hippocampus.

Nicotine has been reported to elicit improvements in cognitive function.[94] These effects may be related to stimulation of neurotransmitter systems within areas of the brain that are important for cognitive processing such as the hippocampus.[95] Nicotine has also been reported to enhance learning and memory in experimental animals which has also been related to increased neural cell genesis in the dentate gyrus of the brain hippocampal formation.[49]

The result of the present study indicates that sub-acute administration of nicotine (animals administered 2mg/kg of body weight nicotine) for seven days showed increased number of pyramidal and granular neurons which was elevated and evident. This finding was in accordance with a study by Carlson *et al.* (2000), who demonstrated that model animals administered low dose of nicotine at 1.70mg/kg nicotine treated for 5 days produced no neuronal degenerative changes.[96]Reasons for similarity in these two findings could be ascribed to the similarity in the dose, route and duration of drug administration in the present study and the study of Carlson *et al.* (2000).

The administration of nicotine to rats for twenty one (2mg/kg; 4mg/kg; 6mg/kg) days showed increased cellularity of the hippocampal neurons with maintained cytoarchitectural and histoarchitectural features in the dentate gyrus and cornus ammonis. This result further revealed that nicotine preserved tissue stroma by attenuating neurodegeneration which was seen in glial cells response to nicotine in the brain. In addition, the resultant pattern also provided and agrees with studies that revealed acute administration of nicotine will invariably lead to neuroprotection as has been previously documented.[27, 97]

Higher doses of nicotine have previously been shown to produce selective degeneration in the brain.[54] The current study displayed a dose and time dependant progressive increase in neuronal population which suggests severe hyperplasia. However, this study also demonstrated that chronic administration of nicotine led to increase cell proliferation rather than neurodegeneration as noted by Adeniyi and Musa (2011), Ijomone *et al.* (2015).[50, 53] Possible reasons for the dissimilarity could be attributed to difference in mode of nicotine administration. In the current study, nicotine was administered orally which implied it was actively metabolized in the liver causing an unchanged fraction of the drug to pose a significant effect. Furthermore, Ijomone et al. in 2015, used the subcutaneous route which implied that nicotine had a slower absorptive rate as compared to the oral route of drug administration.[53] For further studies as regards to this study, electron microscopy is advised as it investigates the ultrastructure of tissues.

Quantitative analysis of the effect of nicotine in hippocampus

Stereological study showed that the pyramidal and granular neurons cell count had a significant cell population with increase cellularity. The cell diameter showed large cell bodies with increased nuclear size of pyramidal cells. This change also

suggests mild hyperplasia and hypertrophy as was already demonstrated in the histomorphology of rats' hippocampus administered nicotine for seven days. Adeniyi and Ogundele in 2014, reported decrease in pyramidal cell layer and granular cell layers on administration of nicotine.[98] Possible reasons for the differences may be due to differences in stereological techniques used. The current study employed the use of the dissector principle which involved the use of counting frames compared to the technique adopted by Adeniyi and Ogundele who measured cell thickness with a method described by World Health Organization in 1991.[87] A more recent stereological principle such as the fractionator principle should be applied in further studies due to its varying range of cell estimation

The pyramidal and granular layers of animals administered nicotine for 21 days, showed a transient elevation in cell count which was very apparent down the group. This hypothesis further buttresses the fact that acute administration of nicotine, lead to moderate hyperplasia and hypertrophy as previously documented and is in accordance with a work by Adeniyi and Musa in 2011 who concluded that nicotine administration caused hyperplasia in the hippocampus and that of Ijomone et al. (2015), who reported hippocampal hypoplasia.[50, 53] The reason for this is likely the time of administration of nicotine. In the index study, different doses of nicotine was administered 12 hours daily this was to maintain a steady and continuous concentration of nicotine in blood circulation as compared to Ijomone et al. (2015), who only administered nicotine once daily.[53]

Hippocampal neuronal cell count of animals administered nicotine for 42 days showed a significant increase in cellularity of pyramidal, granular neuron and pyramidal cell thickness. This contradicts the fact that long chronic exposure of nicotine causes memory deficits and unconsciousness. Possible reasons for this finding could be attributed to the drug receptor interaction between nicotine and ±7nAChRs that resides in the hippocampus. Nicotine has been shown to act on hippocampal α7nAChRs which has been strongly evaluated in learning and memory,[99] and also hippocampal α7 nicotinic receptors have been confirmed to be a viable therapeutic targets.[100] However, further studies should investigate the interaction between nicotine and hippocampal receptors with the use electroencephalogram.

Findings from this study are imperative in the management of Alzheimer's disease (AD). AD is the major causative factor for dementia and it becomes apparent with alterations in mood and behavior, progressive disorientation, memory loss and aphasia which indicate severe cortical dysfunction associated with hippocampal degeneration.[68] Evidence has suggested that accumulation of a peptide (β amyloid) in the brain initiates a chain of events that results in morphological changes in Alzheimer disease. This study recorded an increase proliferation of pyramidal and granular neuron and as such agrees to the assertionthat nicotine is putative in the treatment of the cognitive function experienced by patients with conditions such as AD.[101] Mechanism for this has been demonstrated in both in-vivo and in-vitro studies where nicotine increased the number of nAChRs to attenuate the cognitive deficits found in AD associated with a decrease in the level of acetylcholine due to degeneration of cholinergic neurons.[102, 103] Findings from this study also demonstrated increased and abundance of oligodendrocytes (Gliosis). Gliosis has been described as a nonspecific reactive change of glial cells in response to damage to the central nervous system.[104]

Conclusion

Sub-acute, acute and chronic administration of nicotine showed mild, moderate and severe hyperplasia which was further established by quantitative analysis. Hypertrophy in cell diameters in three key regions of the brain; cerebellum, substantia nigra and hippocampus was also observed. These features were probably enhanced by the drug receptor interaction of nicotine with nAChRs in brain tissue.

Declaration

The authors declare that there was no conflict of interest in the design or preparation of this article.

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