

Decreased density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex of Sprague-Dawley rats after being exposed to filtered kretek cigarette smoke

David Tjahyadi,¹ Edy Parwanto,² Husnun Amalia,³ Reza Aditya Digambiro,⁴ Hosea Jaya Edy,⁵ Ashaolu Victoria Oladimeji⁶

¹Department of Histology; ²Department of Biology; ³Department of Ophthalmology; ⁴Department of Anatomical Pathology, Faculty of Medicine, Universitas Trisakti, Indonesia; ⁵Study Program of Pharmacy, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Indonesia; ⁶Department of Chemistry, Loyola Institute of Frontier Energy, Loyola college, Chennai, India

Correspondence: David Tjahyadi, Department of Histology, Faculty of Medicine, Universitas Trisakti, Jl. Kyai Tapa, Kampus B, No.260 Grogol 11440, Jakarta-Indonesia.
Email: davesaboch@trisakti.ac.id

Key words: cerebral cortex; filtered kretek cigarette smoke; pyramidal cell; cerebellar Purkinje cell.

Acknowledgements: we express our deep gratitude to the Leaders and Staff of the RSHP Laboratory of the Faculty of Veterinary Medicine, IPB-University, Bogor, Indonesia for facilitating in this study.

Conflicts of interest: the authors declare that they have no competing interests.

Funding: this research was funded by the Faculty of Medicine, Universitas Trisakti, for the 2021-2022 fiscal year, No. 5917/USAK-TI/FK/03/XII/2021.

Contributions: conceptualization: DT, EP, and HA. Data acquisition: DT, and EP, Data analysis or interpretation: EP, HA, HJE. Drafting of the manuscript: DT, EP and HA. Critical revision of the manuscript: EP, HA, HJE, RAD, and AVO. Approval of the final version of the manuscript: all authors.

Ethics approval: this research has received a certificate of passing the ethical review from the Research Ethics Commission of the Faculty of Medicine, Universitas Trisakti, with No: 184/KER/FK/VIII/2018.

Received: 23 July 2022.

Accepted: 14 March 2023.

Early view: 13 April 2023.

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

©Copyright: the Author(s), 2023

Licensee PAGEPress, Italy

Journal of Biological Research 2023; 96:10757

doi:10.4081/jbr.2023.10757

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial International License (CC BY-NC 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Abstract

Filtered kretek cigarette smoke is a gas that contains solid components (particulates). Carcinogenic chemicals are present in this type of cigarette smoke. Furthermore, it is said that tobacco has a major negative impact on cerebral structure development, creates addiction, and alters brain activity and function. The purpose of this study was to see how filtered kretek cigarette smoke affected the density of pyramidal cells in the cerebral cortex and Purkinje cells in the cerebellar cortex. An experimental and control group design was used for the study. Group 1 (6 rats) is the control group, and the rats breathe normally. Group 2 (6 rats) was exposed to filtered kretek cigarette smoke at a rate of one stick per day for three months. Pyramidal cells in the rat cerebral cortex and Purkinje cells in the cerebellar cortex were studied. Pyramidal cells were expected to be distributed in the cerebral cortex at the hippocampus area of rats in group 1. In contrast, the density of pyramidal cells decreased in group 2. The number of pyramidal cells in the rat cerebral cortex differed significantly between groups 1 and 2 ($p < 0.001$). Purkinje cells in the cerebellar cortex in group 1 were normal, whereas Purkinje cells in group 2 were degenerated. The distance between Purkinje cells in the cerebellar cortex was greater in group 1 than in group 2 ($p < 0.001$). Rats exposed to 1 stick of Kretek cigarette smoke each day for 3 months had lower pyramidal cell density in the cerebral cortex and hippocampus. The same result happened: one stick of Kretek cigarette smoke each day for three months reduced Purkinje cell density in the cerebellar cortex.

Introduction

Smoking globally causes considerable health problems. Around 2.5 billion people in the world are smokers and that number even greater is in third-world countries. Indonesia's population is included in the group of third-world countries, meaning that a consistent portion of the population consumes cigarettes.¹

Cigarette smoke is a gas that contains solid materials (particulates), including carcinogenic ingredients. Moreover, it is stated that tobacco has a significant negative impact on the development of nervous structures² and causes addiction.³ Gases and particulates of cigarettes are channeled into the alveoli.⁴ It is also stated that smok-

ing affects the activity and function of the brain and interferes with the psychology of cigarette addicts.⁵

The cerebral cortex is the brain's largest and most visible component; it is separated into right and left hemispheres by a deep groove or fissure known as the major longitudinal fissure.⁶ The cerebral cortex's surface is extended by multiple gyri, which are elevations separated by grooves called sulci.⁷ Neurons in the cerebral cortex have pyramid-shaped cell bodies.⁸ The cerebral cortex is grey matter on the outside of the cerebral hemispheres, and the centre of the medulla is white matter on the inside of the cerebral hemispheres.⁶ Myelinated and unmyelinated fibres, oligodendrocytes, fibrous astrocytes, and microglial cells are all found in white matter. Perikaryon or nerve cell bodies, unmyelinated fibres, myelinated fibres, astrocytes, oligodendrocytes, and microglial cells are all found in grey matter. The quantity of myelinated nerve fibres is indicated by the unique colour of the white matter.⁷

The cerebral cortex serves as a hub for learning, memory, sensory integration, information processing, and motor response initiation. This structure is made of grey matter and contains an estimated 10 billion nerve cells.⁹ Efferent pyramidal cells are the most common nerve cells and can be seen under a microscope.¹⁰

The results of previous studies showed that cigarette smoke is a risk factor for the occurrence of aneurysms in the brain (cerebral aneurysms). It has also been reported that cigarette smoke increases the risk of rupture.^{11,12} It was further stated that more than 80% of patients with Aneurysmal Subarachnoid Hemorrhage (ASH) had a history of smoking, and 50–60% were smokers.¹¹ So far, it is not known how filtered kretek cigarette smoke affects the biometrics of brain cells.

Previous research has shown the importance of cell biometrics as a characteristic that needs to be developed to assist in early diagnosis at the cellular and tissue levels.^{13,14} This needs to be done so that the diagnosis related to cell abnormalities is determined more objectively based on the quantitative data obtained and not only qualitative data. The brain is the focus of research because of the importance of this organ in the regulation of body activities. In addition, parts of the brain, namely the cerebral cortex and cerebellum, are vulnerable to exposure to foreign substances.^{15,16} Cigarette smoke mostly contains alkaloids. The results of previous studies have shown that cigarette smoke is associated with decreased cognitive function and causes dementia. More precisely, cigarette smoke causes thickening of the cortex in the brain.¹⁷ Another study has demonstrated that cigarette smoke inhibits mitochondrial respiration in the rat brain.¹⁸

To explore more deeply the effect of cigarette smoke on the density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex, we used Sprague-Dawley rats as animal models. Our recent research demonstrates the importance of cell biometrics as a characteristic that needs to be developed to aid early diagnosis at both cellular and tissue levels.^{19,20}

Materials and Methods

Research design

This study used *Rattus norvegicus*, Sprague-Dawley strain, to test the effect of filtered kretek cigarette smoke on pyramidal cells in the cerebral cortex and Purkinje cell density in the cerebellar cortex. This study has two groups, the treatment group and the control group, thus adopting an experimental and control group design.

Location and time of research

Experimental animal treatment was carried out at the RSHP Laboratory of the Faculty of Veterinary Medicine, IPB-University, Bogor, Indonesia. Histological observations of the preserved preparations of rat brains (cerebral and cerebellar cortex) were carried out at the Biology Laboratory, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia. This research was conducted between September 2021 to June 2022. This research has received a certificate of passing the ethical review from the Research Ethics Commission of the Faculty of Medicine, Universitas Trisakti, with No: 184/KER/FK/VIII/2018.

Selecting and grouping of experimental animals

Rattus norvegicus Sprague-Dawley strain rats, aged 2-3 months with a body weight of 200-300 g, were included in the study; the exclusion criteria were if the animals died during treatment. The number of samples is estimated in accordance with World Health Organization (WHO) guidelines, which indicate that at least 5 animals must be utilized in experimental research. Each set of six rats was employed in this study. The control group is group 1, while the treatment group is group 2.

Treatment of experimental animals

Rats were acclimatized for one week before the trial began. During acclimation, rats were provided food and water. Rats were not treated during acclimation. After acclimation, the groups were randomised at random. Group 1 (6 rats) is the control group, and the rats breathe normally. Group 2 (6 rats) was exposed to filtered kretek cigarette smoke 1 stick every day for 3 months (90 days).

Filtered kretek cigarette smoke exposure was carried out in a smoking chamber measuring 45 x 35 x 20 cm (31,500 cm³). The oxygen valve was opened, then the cigarette was attached to the pipe connected to the pump, then the cigarette was burned and the pump was turned on so that the smoke will enter the smoking chamber and be inhaled by the rats. Filtered kretek cigarette smoke exposure was carried out on group 2 for 10 minutes every day in the morning.

The rearing cage measures 482 x 267 x 210 mm (27025740 mm³=27025.740 cm³). The treatment cage room was equipped with air conditioning with a temperature of 22 ± 3°C, humidity 55 ± 5%, and LED lights (12:12 hours, light and dark cycle), feed and drink in normal amounts (reasonable). The standard feed used for rats was brailler-II pellet (BR-II) containing corn, soybean meal, wheat pollard, coconut meal, fish meal, meat meal, rice flour, tapioca, coconut oil, and fish oil premix. Feed and water were provided *ad libitum*. Feed was given as much as 10% body weight (± 10-15 grams/day), every day in the morning and evening. Drinking water for rats was always changed every day.

After treatment, the rats were anesthetized until they died. Anesthesia was administered using ketamine 100 mg/kg body weight and xylazine 10 mg/kg body weight intra peritoneally. After the treatment, the experimental animals were sacrificed by euthanasia, then their organs, namely the brain, were taken. To take the rat brain, decapitation was carried out, then the skull and brain were separated. The brain that had been separated from the skull, was then put into a small pot containing 10% neutral buffered formalin (NBF).

Observation of rat cerebral and cerebellar tissues

The thickness of the cerebral tissue, as well as the cerebellar prepared for making slides, was 3 mm. Rat cerebral tissue, as well as rat cerebellar tissue in paraffin blocks were cut with a thickness of 5 µm. Next, the tissue sections were stained with hematoxylin and

eosin (HE). Observations were made on pyramidal cells in the cerebral cortex, in the hippocampus area. In addition, Purkinje cells were also observed in the gray matter of cerebellar cortex. Observation of pyramidal cells and Purkinje cells was carried out by 3 observers according to predetermined guidelines.

Data analysis

The statistical test chosen in this study was an independent statistical test T-test to compare the histometric brain cells of rats between the group 1 and the group 2. Differences between groups were significant if the results of the analysis showed a p value <0.05.

Results

Brain morphology of Sprague-Dawley rats and sites of tissue sectioning for preparation of slides from the cerebral and cerebellar tissues are presented in Figure 1.

Photomicrograph of rat cerebral cortex in group 1 and group 2 are presented in Figure 2, while a comparison of the density of pyramidal cells in the cerebral cortex of rats in the hippocampal area between groups 1 and group 2 is presented in Figure 3.

Based on Figure 3 above, the distribution of pyramidal cells in the rats cerebral cortex at the hippocampus area of group 1 was normal, while group 2 showed a decrease in pyramidal cell density. Comparison of pyramidal cell indices in the rats cerebral cortex in the hippocampus area between group 1 and group 2 is presented in Figure 4.

The density of pyramidal cells in the rats cerebral cortex at the hippocampus area between the group 1 was compared to group 2 (p<0.001). The result of statistical analysis showed that t value=22.262; df=118; mean difference=7.60983; SE diff.=0.34182; 95% CI=6.93293-8.28674.

Photomicrograph of the rats cerebellar cortex is presented in Figure 5 and Figure 6.

Purkinje cells in the rats cerebellar cortex in the group 1 seemed normal, while in the group 2 they appeared degenerated. Comparison of Purkinje cell distances in the rat cerebellar cortex between the group 1 compared to the group 2 is presented in Figure 7.



Figure 1. Brain morphology of Sprague-Dawley rats and sites of tissue sectioning for preparation of slides from the cerebral and cerebellar cortex. Red circle, bregma; white circle, lambda; black line, tissue cutting site for making cerebral slides; red line, tissue cutting site for making cerebellar slides.

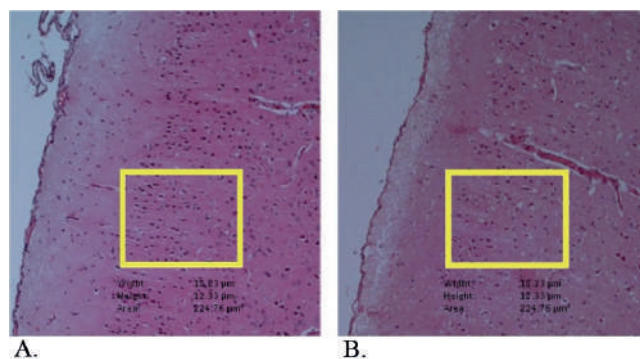


Figure 2. Photomicrograph of rat cerebral cortex, stained with hematoxylin and eosin (objective 10 X). Yellow rectangles are used to show the density of pyramidal cells. A. Cerebral cortex of rat in group 1. B. Cerebral cortex of rat in group 2.

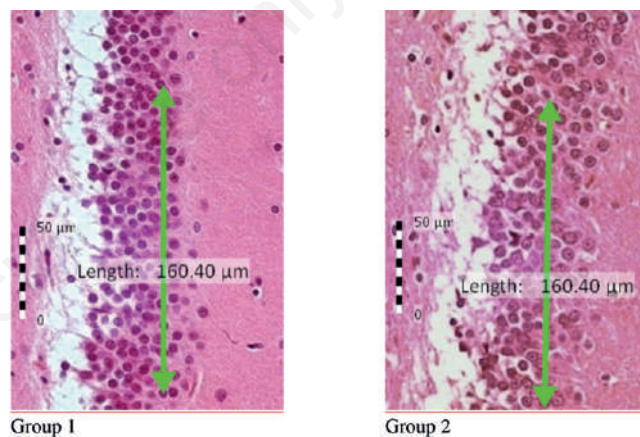


Figure 3. Hippocampus area of cerebral cortex in rat. A. Hippocampus area of cerebral cortex in group 1, stained with hematoxylin and eosin (objective 40 X). B. Hippocampus area of cerebral cortex in group 2, stained with hematoxylin and eosin (objective 40 X).

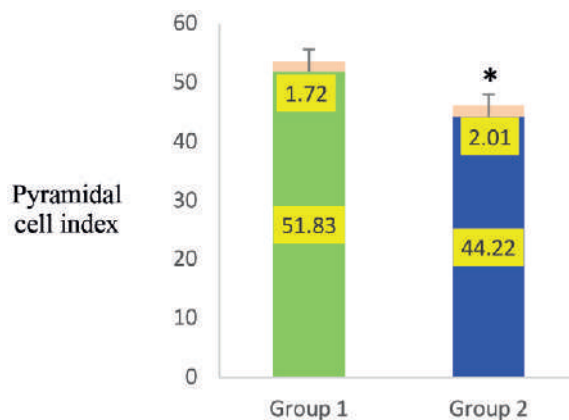


Figure 4. Comparison of pyramidal cell index in the hippocampus area of the rat cerebral cortex. *Significant difference (p<0.001).

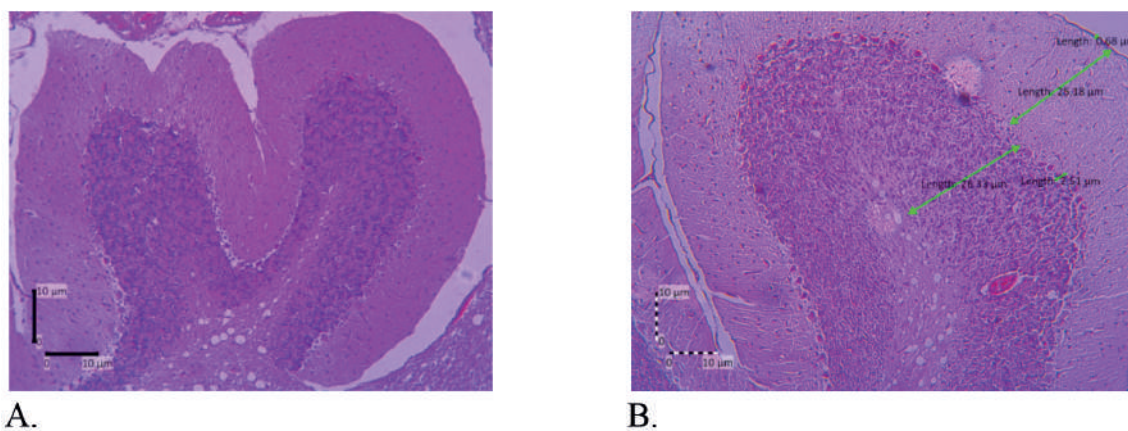


Figure 5. Photomicrograph of rats cerebellar cortex. A. Photomicrograph of rat cerebellar cortex, stained with hematoxylin and eosin, objective 4 X. B. Photomicrograph of rat cerebellar cortex, stained with hematoxylin and eosin, objective 10 X.

Based on Figure 7, the distances of Purkinje cells in the rats cerebellar cortex of the group 1 were more dense than those in the group 2. There were differences in the distance of Purkinje cells in the rats cerebellar cortex between group 1 compared to the group 2 ($p < 0.001$). The result of statistical analysis showed that t value = 29.263; $df = 118$; mean diff. = 1.38517; SE diff. = 0.04733; 95% CI = 1.47890 to 1.29143.

Discussion

Alkaloids are found in tobacco. Cigarettes on the market contain approximately 1.5% alkaloids by weight of tobacco. Tobacco, the primary component of cigarettes, accounts approximately 95% of the overall alkaloid content.²¹ Nicotine ingested when smoking travels quickly to the brain. A prior study found that 2 mg of nicotine was absorbed systemically after 20 minutes of smoking and 80 puffs using a nicotine inhaler device.²² In this study, filtered kretek cigarette smoke exposure can impact brain structure and function. Our study's approach of cigarette smoke exposure is similar to other studies.²³ It is indeed difficult to arrange for each rat to be exposed to the same amount of cigarette smoke. This can be remedied by repairing the smoking box.

Photomicroscopy of the cerebral cortex from the frontal lobes of rats revealed the pia mater, which encloses the molecular layer, followed by external granular, external pyramidal, internal granular, internal pyramidal, and a polymorphic innermost layer. Group 1 had a higher density of pyramidal cells in the exterior granular layer of the hippocampus than Group 2. We calculated the pyramidal cell density, often known as the pyramidal cell density index. This is accomplished by dividing the number of pyramidal cells by the length of a line passing through the exterior granular layer. The same study found that the control group had normal histology in the cerebral cortex layer, whereas tramadol administration caused pyramidal cell degeneration. Furthermore, granular cells with pyknotic nuclei and the formation of haloes.^{24,25} Previous research found that tramadol administration caused histological abnormalities and apoptosis in the cerebral cortex, which was linked to oxidative stress.²⁶ More specifically, the onset of apoptosis is preceded by damage to the DNA structure.²⁷

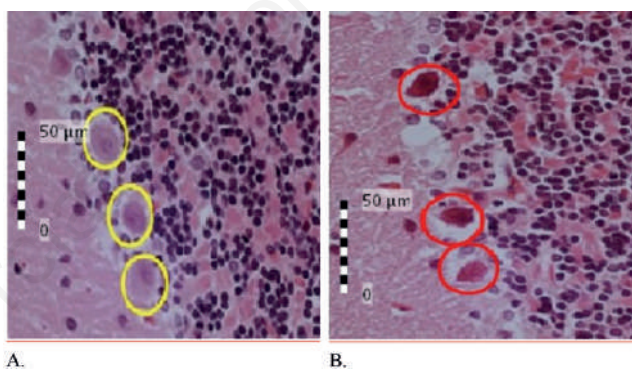


Figure 6. Histological appearance of the rat cerebellar cortex. A. Rats cerebellar cortex in the group 1, stained with hematoxylin and eosin, objective 40 X. B. Rats cerebellar cortex in the group 2, stained with hematoxylin and eosin, objective 40 X. Purkinje cells in the rat cerebellar cortex in the group 1 looks normal (yellow circle), while in the group 2 there is a picture of degenerated Purkinje cells (red circle).

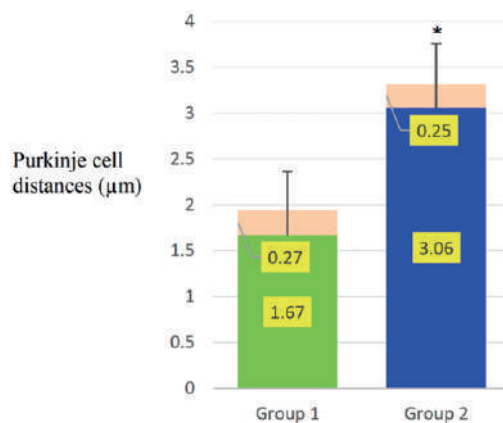


Figure 7. Comparison of Purkinje cell distances in rat cerebellar cortex. *Significant difference ($p < 0.001$).

Pyramidal cells in the rat cerebral cortex are easily recognized as the cells are quite large and in large numbers. In group 1, pyramidal cells in the rats' cerebral cortex appeared normal with high density. The density of pyramidal cells in the rats' cerebral cortex was lower in group 2 compared than the group 1. The results of previous studies demonstrated that pyramidal cell death can be caused by hypoxia,^{28,29} so that the cells experience cell hemostatic disorders.³⁰

Purkinje cells are found in the ganglion layer of the cerebellar cortex. Purkinje cells in the ganglion layer of the rats' cerebellar cortex seemed to be conical in form in group 1. Purkinje cells in the ganglion layer of the rat cerebellar cortex in group 2 were deteriorated and necrotic, according to our findings. Also, the vascularization is becoming increasingly thick. We believe this is owing to compensatory degeneration and necrosis, which is accompanied by enhanced anastomotic perfusion. According to the findings of this study, the position of Purkinje cells in the ganglion layer of the rats' cerebellar cortex in groups 1 and 2 did not change. This means that the Purkinje cells are still in the correct location in the ganglion layer. Purkinje cells in the ganglion layer of the rats' cerebellar cortex in group 2 were obviously pyramidal in shape, indicating degeneration and necrosis. It is widely assumed that cerebellar cognitive function involves interactions between the cerebellar cortex and cerebral cortex association areas.³¹

The cerebellar cortex is susceptible to toxic chemicals such as nicotine. Nicotine can cause Purkinje cells to experience mild, moderate and severe hyperplasia depending on the dose, time and method of administration.³² Our findings contradict prior research that found nicotine to be protective in the cerebellar granule cells.³³ When rats were given nicotine for seven days, they developed modest hyperplasia compared to rats given nicotine for 21 and 42 days. Furthermore, it was indicated that exposure to modest dosages of nicotine for a brief length of time promoted neuronal cell growth.³⁴

Our results are in line with previous studies were reported that nicotine causes neurodegeneration in the cerebellar cortex. This is due to the administration of higher doses of nicotine and a longer administration time.³⁵ Our results are also in agreement with previous studies which demonstrated that Purkinje cells undergo neurodegeneration due to exposure to cigarette smoke in rats.³⁶ It should be noted from the results of previous studies that low doses of nicotine have a stimulatory effect on the central nervous system.³⁷ The findings of our study add to the evidence that low-dose filtered kretek cigarette smoke is harmful to the brains of Sprague-Dawley rats.

Despite the fact that we did not measure the levels of chemical compounds in filtered kretek cigarette smoke, which is a limitation of this study, the facts show that pyramidal cells in rats' cerebral cortex appear abnormal and have decreased in density. Furthermore, it was shown that Purkinje cells degenerate and their cell density decreases in the rat cerebellar cortex. Furthermore, we did not perform immunohistochemical analysis as a specific marker of various cytotypes in the cerebral and cerebellar cortex of rats.

Conclusions

According to the study's findings, rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months reduced the number of pyramidal cells in the cerebral cortex around the hippocampus. Exposure to filtered kretek cigarette smoke at a rate of 1 stick/day for 3 months reduced Purkinje cell density in the cerebellar cortex.

References

1. World Health Organization. WHO report on the global tobacco epidemic 2021. World Health Organization: New York 2021. Available from: <https://www.who.int/publications/i/item/9789240032095>
2. Hajdusianek W, Żórawik A, Waliszewska-Prosól M, et al. Tobacco and nervous system development and function-new findings 2015–2020. *Brain Sci* 2021;11:1-15.
3. Mishra A, Kumar R, Mishra SN, et al. Nicotine addiction and psychological stress: a case-control study among the unemployed North Indians. *Res Square Preprint* 2022;1-19.
4. Sahu SK, Tiwari M, Bhangare RC, Pandit GG. Particle size distributions of mainstream and exhaled cigarette smoke and predictive deposition in human respiratory tract. *Aerosol Air Qual Res* 2013;13:324-32.
5. Liem A. Pengaruh nikotin terhadap aktivitas dan fungsi otak serta hubungannya dengan gangguan psikologis pada pecandu rokok. [The influence of nicotine on activity and brain function and its relationship with psychological disorders of cigarette addicts] *Bul Psikol* 2010;18:37-50.
6. Lorimer A. The cerebrum, its hemispheres and structures. Preprint 2020, Available from: https://www.researchgate.net/publication/347885443_The_Cerebrum_its_hemispheres_and_structures
7. Donkelaar HJ, Tzourio-Mazoyer N, Mai JK. Toward a common terminology for the gyri and sulci of the human cerebral cortex. *Front Neuroanat* 2018;12:1-17.
8. Shreejha MV, Priyadarshini R, Sinduja P, Meghashree V. Analysis of histological features of the cerebral cortex and hippocampus of albino rats using haematoxylin & eosin stain-an observational study. *JPRI* 2021;33:53-64.
9. Yang L, Li M, Zhan Y, et al. The impact of ischemic stroke on gray and white matter injury correlated with motor and cognitive impairments in permanent MCA Orats: A multimodal MRI-based study. *Front Neurol* 2022;13:1-18.
10. Ilbay G, Balıkcı A, Köktürk S, et al. Neonatal tactile stimulation downregulates dendritic spines in layer V pyramidal neurons of the WAG/Rij rat somatosensory cortex. *Neur Plasticity* 2022; 7251460:1-9.
11. Chalouhi N, Ali MS, Starke RM, et al. Cigarette smoke and inflammation: Role in cerebral aneurysm formation and rupture. *Med Inflamm* 2012;271582:1-12.
12. Woo D, Khoury J, Haverbusch MM, et al., Smoking and family history and risk of aneurysmal subarachnoid hemorrhage. *Neurology* 2009;72:69-72.
13. Yúfera A, Olmo A, Daza P, Cañete D. Cell biometrics based on bio-impedance measurements. *Adv Biometric Tech* 2011;17: 343-366.
14. Tjahyadi D, Parwanto E. Applications of biometrics, histometrics and cytometrics in deep learning. *Bali Med J* 2022, 11(2): 520-522.
15. Bakar B, Kose EA, Sari E, et al. Effects of low dose methotrexate in cerebral ischemia reperfusion injury in rat. *J Neurol Sci [Turk]* 2015;32:507-18.
16. Srivastava A, Liachenko S, Sarkar S, et al. Global neurotoxicity: Quantitative analysis of rat brain toxicity following exposure to trimethyltin. *Int J Toxicol* 2021;40:367-79.
17. Karama S, Ducharme S, Corley J, et al. Cigarette smoking and thinning of the brain's cortex. *Mol Psychi* 2015;20:778-85.
18. Lee HM, Hallberg LM, Greeley GH Jr., Englander EW. Differential inhibition of mitochondrial respiratory complexes

- by inhalation of combustion smoke and carbon monoxide, *in vivo*, in the rat brain. *Inhal Toxicol* 2010;22:770-7.
19. Parwanto MLE, Wratsangka R, Guyansyah A, Anggraeni K. Mutation of the fas-promoter-670 gene, AA to GA in the normal cervix-epithelial-cells of high risk Indonesian mother: A case report. *Bali Med J* 2019;8:360-4.
 20. Parwanto E, Wratsangka R, Guyansyah A, et al. The change of cell biometric and its nucleus on cervical-squamous-epithelial-cell with GA genotype of Fas-promoter-670 gene, high-risk human papillomavirus and Candida species infection: a case report. *Bali Med J* 2021;10:74-81.
 21. Rodgman A, Perfetti TA. The chemical components of tobacco and tobacco smoke. 2013, Second edition. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group.
 22. Rostami AA, Campbell JL, Pithawalla YB, et al. A comprehensive physiologically based pharmacokinetic (PBPK) model for nicotine in humans from using nicotine containing products with different routes of exposure. *Sci Reports* 2022;12:1-18.
 23. Alkhatib AJ, Ababneh SK. The impacts of cigarette smoking on rat's trachea: a histologic study. *Biomed J Sci & Tech Res* 2021;38:30006-10.
 24. Mahmoud ES, Al-Shahed FA-ZN, Ouda EA, Al Anany MG. Effect of thymoquinone on the structure of the cerebral cortex of adult male albino rats treated with tramadol. *Sci JAI-Azhar Med Fac Girls* 2019;3:97-110.
 25. Motawea SM, Amer RM, Haiba DA, Mostafa MS. Cerebral cortical changes in adult albino rats under the effect of tramadol and its withdrawal: histological and morphometric study. *Egypt J Histol* 2020;43:412-26.
 26. Ghoneim FM, Khalaf HA, Elsamanoudy AZ, Helaly AN. Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. *Int J Clin Exp Pathol* 2014;7:7323-41.
 27. Saleh EM, Hamdy GM, Hassan RE. Neuroprotective effect of sodium alginate against chromium-induced brain damage in rats. *PLoS One* 2022;17:e0266898.
 28. Zhuravin IA, Dubrovskaya NM, Vasilev DS, et al. Prenatal hypoxia produces memory deficits associated with impairment of long-term synaptic plasticity in young rats. *Neurobiol Learn Mem* 2019;164:107066.
 29. Amakhin DV, Soboleva EB, Postnikova TY, et al. Maternal hypoxia increases the excitability of neurons in the entorhinal cortex and dorsal hippocampus of rat offspring. *Front Neurosci* 2022;16:1-12.
 30. Vlachos A, Ikenberg B, Lenz M, et al. Synaptopodin regulates denervation-induced homeostatic synaptic plasticity. *Proc Natl Acad Sci USA* 2013;110:8242-7.
 31. McAfee SS, Liu Y, Sillitoe RV, Heck DH. Cerebellar coordination of neuronal communication in cerebral cortex. *Front Syst Neurosci* 2022;15:781527.
 32. Oyem JC, Odokuma EI. Histomorphological effects of nicotine on selected parts of the brain of adult Wistar rats. *Galician Med J* 2018;25:E2018213.
 33. Tizabi Y, Al-Namaeh M, Manaye KF, Taylor RE. Protective effects of nicotine on ethanol-induced toxicity in cultured cerebellar granule cells. *Neurotox Res* 2003;5:315-21.
 34. Jalili S, Sadeghi Y, Sahraei H. Morphological changes in hippocampus CA1 neurons after nicotine administration in rats. *J Kermanshah Univ Med Sci* 2009;13:e79823.
 35. Lokanadham S, Kothandaraman U, Chidambaram R, et al., Long term administration of nicotine in albino rat cerebellum-histopathological study. *Int J Pharm Sci Rev Res* 2015;34:114-7.
 36. Omotoso GO, Adekeye MO, Ariyo AA, et al. Neurohistochemical studies of adolescent rats' prefrontal cortex exposed to prenatal nicotine. *Ibnosina J Med and Biomed Sci* 2014;6:25-30.
 37. Matta SG, Balfour DJ, Benowitz NL, et al. Guidelines on nicotine dose selection for *in vivo* research. *Psychopharm* 2007;190:269-319.