ISSN: Print: 2811-1672
Online: 2811-1680

www.njbams.asmeda.org

NJBAMS

Nigerian Journal of Basic and Applied Medical Sciences

An official publication of the National

ASSOCIATION OF SPECIALIST MEDICAL DOCTORS IN ACADEMICS (ASMeDA aka MEDSABAMS)

Research Article *https://doi.org/10.53994/NJBAMS.202111.2*

Histological Changes of Wistar Rats' Hippocampus and Entorhinal Cortex After Prenatal Exposure to Mosquito Coil Smoke

Shehu K[1], Sirajo M.U.[2], Saleh M.S.[2]

[1]Department of Anatomy, College of Health Sciences, Federal University, Birnin-Kebbi
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano

ABSTRACT

Background: The structural integrity of the hippocampus and the entorhinal cortex appears to be a prerequisite for normal acquisition of information about relational and contextual representation. Increased exposures to pyrethroids by pregnant women and children have raised concerns over their potentials as developmental neurotoxicants.

Objectives: We studied the histological changes on the hippocampus and entorhinal cortex of adolescent Wistar rats prenatally exposed to mosquito coil smoke (MCS).

Methods: 30 adult Wistar rats (20 females, 10 males) were used for the study. Mating was induced, and pregnancy was confirmed. Pregnant animals were grouped into four, 3 animals per group. Group I was exposed to fresh air. Groups II, III, and IV were exposed to mosquito coil smoke for 4, 6 and 8 hours daily respectively throughout gestation period. On Post-natal day (PND) 29, experimental animals were humanely sacrificed and regions of the hippocampus and entorhinal cortex were processed for histological studies using H & E stain.

Results and Conclusion: Our results showed that prenatal exposure to mosquito coil smoke caused neuronal degeneration, distortion in cyto-architecture of cellular layers and vacuolations in the hippocampus and entorhinal cortex of prenatally exposed groups.

Keywords: Memory, Entorhinal cortex, Histomorphology.

Corresponding Author:

Name: Saleh MS,
Address: Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria.
Email: sagirsaleh007@gmail.com.
Phone: 08023098344.

INTRODUCTION

The developing brain contains low antioxidant defense and thence, is more susceptible to oxidative insults (1). Damage to a particular structure or to connecting pathways in the developing nervous system can produce structural or functional changes that result in behavioral deficit (2,3,4). The hippocampus is an important structure of the medial temporal lobe, and its structural integrity is crucial for normal acquisition of spatial and relational processes (5,6). The entorhinal cortex is the primary connection between the hippocampus and neocortex and function in processes of recognition and contextual memory representations as well (5,7).

Inhalation of mosquito repellents during prenatal life may have key role in inducing abnormalities and oxidative stress by decreasing antioxidants such as Reduced Glutathione, Catalase, Superoxide dismutase, Ascorbate, Zinc e.t.c in the developing central nervous system of rat pups (1,8). The increased oxidative stress can alter bloodbrain barrier (BBB) as well as neurotransmitter and their associated receptors (9-11). Histopathological malformations such as cleft palate and necrotic brain have been observed in rat offspring following maternal exposure to pyrethroids (12). The neurotoxicity of pyrethroids pesticides and their teratogenic potentials have been reviewed extensively in earlier studies (13,14). The active ingredient commonly found in mosquito coils is Allethrin or Meperfluthrin (19). Some others may contain pyrethrin, Cyfluthrin, or Cypermethrin (19).

Despite its health implications, mosquito coils are still being used by households in Nigeria to control mosquitoes, including during pregnancy and lactation. Therefore, it is important to investigate its effect on the neurohistology of prenatally exposed offspring.

Cite this article as: Shehu K., Sirajo M.U., Saleh M.S. Histological Changes of Wistar Rats' Hippocampus and Entorhinal Cortex after Prenatal Exposure to Mosquito Coil Smoke. Nig. J. Basic & Applied Med. Sci. 2021;1(1);7-09.

METHODOLOGY

Ethical Clearance

Approval for this study was obtained from the research ethical committee of Anatomy Department, Faculty of Basic Medical Sciences, Bayero University Kano.

Materials

Mosquito coil (Brand Name: WAVETIDE, Active Ingredient: 0.05% Meperfluthrin, Pesticide Registration Number: WP20140093, Production Certificate Number: HNP 33099-I2694), partially ventilated chamber (2.00 x 0.98 x 1.55m), Wistar rats (20 females, 10 male), Animal feed (Olam feeds), Light Microscope (Nikon E600 microscope, U.S.A), Digital AmScope (MD 900, Japan).

Animals, Mating and Confirmation of Pregnancy

A total of 30 adult Wistar rats of about 90 days old (20 females, 10 males) with average weight of 180g were used for the study. The animals were kept at the animal house of Department of Anatomy, Bayero University Kano under standard animal house conditions. Animals were housed in plastic cages with wire gauze at the top. They were fed with grower mash and water *ad Libitum*. A 12 hour day/night cycle was maintained at room temperature. Animals were allowed to acclimatize for two weeks. Female rats were allowed to mate with males in a ratio of 2:1. The presence of vaginal plug indicates the occurrence of mating (15). The day that vaginal plug was detected was designated as day 1 of gestation (15). 12 pregnant rats were randomly grouped into four, 3 animals per group and then transferred into separate unit cages.

Exposure to Mosquito Coil Smoke

Animal groups in their respective cages were placed in a partially ventilated chamber (2.00 x 0.98 x 1.55m in dimensions) and a window cut on two sides of the exposure chamber were made to allow for ventilation (16). For each of the groups, one mosquito coil was weighed, and the required gram was lit and placed in the

exposure chamber.

Table 1. Grouping of Pregnant Animals

Group	Dose
Group 1	Control group exposed to room air throughout gestation.
Group 2	Animals were exposed to mosquito coil smoke for 4 hours daily (6 grams of burning mosquito coil) throughout gestation.
Group 3	Animals were exposed to mosquito coil smoke for 6 hours daily (9 grams of burning mosquito coil) throughout gestation.
Group 4	Animals were exposed to mosquito coil smoke for 8hours daily (12grams of burning mosquito coil) throughout gestation.

Animal Sacrifice

On Post natal day (PND) 30, the animals were anaesthetized using 10mg/Kg ketamine. Subsequently, the animals were perfused (transcardial) through the left ventricle with 10% Formalin. Subsequently, the skull was opened to harvest the whole brain following which it was fixed in the perfusion fixative.

Tissue Processing

After fixation, sections of 5 um thickness were taken through the hippocampus and entorhinal cortex. The tissues were processed and paraffin wax embedded tissues blocks were prepared. Sections were taken and subsequently processed for routine H & E stain. Tissues were then mounted with cover slips using a mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken.

RESULTS

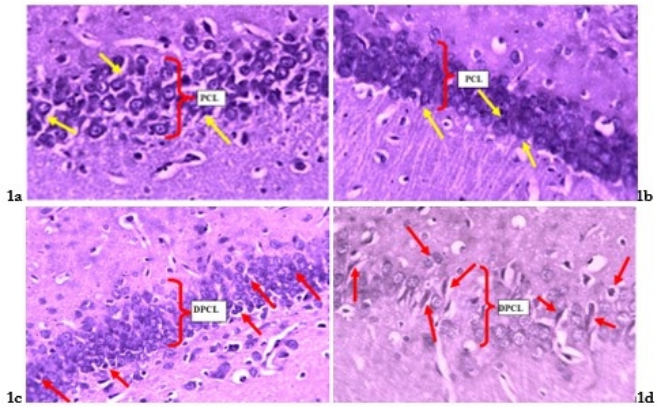


Figure 1a-d: Photomicrograph Showing Sections of the Hippocampus of Adolescent Wistar Rats for Control Group (1a) and Groups Prenatally Exposed to Mosquito Coil Smoke for 4, 6 and 8 Hours Daily Throughout Gestation (1b, 1c and 1d respectively). Figure 1a and 1b Shows Normal Architecture with Pyramidal Cells (Arrow) and the Pyramidal Cell Layer (PCL) Appearing Normal. Figure 1c Shows Distorted Pyramidal Cell Layer (DPCL) With Some Degenerating (Pyknotic) Cells (Arrows). Figure 1d Shows Distorted Pyramidal Cell Layer (DPCL) With Many Degenerating Cells (Pyknosis and Nuclear Shrinkage) (Arrow). (H & E ×250).

DISCUSSION

This study was designed to investigate the histological changes on two important brain regions; the hippocampus and entorhinal cortex in prenatally exposed adolescent Wistar rats following daily gestational exposure to mosquito coil smoke (MCS).

Developmental neurotoxicity involves gross dysmorphology of the nervous system or changes in neurobehavior, neurochemistry and/or neurohistology observed in the offspring, following maternal exposure to xenobiotic during gestation or lactation periods.

Despite its health implications, mosquito coils are still being used by households to control mosquitoes in Nigeria. Studies on the developmental neurotoxicity of MCS are scanty. Previous studies have focused on the effects of prenatal exposure to MCS on

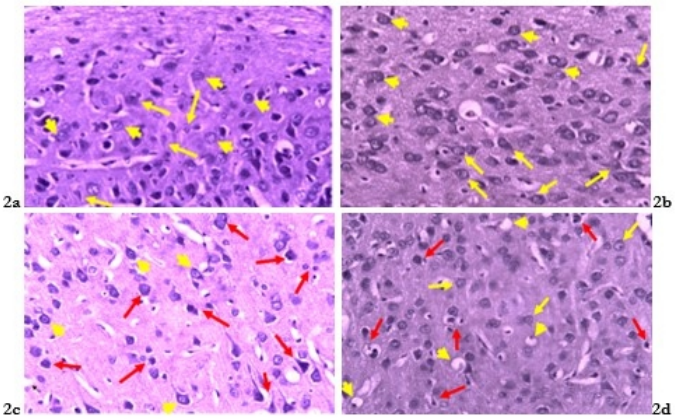


Figure 2a-d: Photomicrograph Showing Sections of the Entorhinal Cortex of Adolescent Wistar Rats for Control Group (2a) and Groups Prenatally Exposed to Mosquito Coil Smoke for 4, 6 and 8 Hours Daily Throughout Gestation (2b, 2c and 2d respectively). Figure 2a and 2b Show Normal Stellate Cells (Short Arrow) and Normal Pyramidal Cells (Long Arrow). Figure 2c Show Degenerating (Eosinophilic) Cells (Arrows) and Some Normal Cells (Arrow Head). Figure 2d Shows Many Degenerating Cells (Pyknosis and Nuclear Shrinkage) (Red Arrow), Vacuolations (Arrow Head) and Some Normal Cells (Yellow Arrow) (H & E ×250).

structures of the reproductive, urinary and respiratory systems. Some studies have reported the developmental neurotoxicity of other forms of mosquito repellants and pyrethroids other than mosquito coils, and have focused more on neurobehaviour and neurochemistry leaving out its effects on neuronal structure. Interestingly, this is the first study to report the effects of mosquito coil smoke on the histology of the hippocampus and entorhinal cortex in prenatally exposed rats' offspring.

In this study, varying degrees of histomorphological changes in the hippocampus and the entorhinal cortex of adolescent wistar rats exposed to MCS during gestation were observed. The MCS exposed groups (III and IV) showed varying forms of histomorphological changes implying some signs of neurodegeneration that could affect the normal functions of the hippocampus and entorhinal cortex. These changes include disruption of the cytoarchitecture of the pyramidal cell layer of the hippocampus (Figure 1c-1d), degeneration of pyramidal cells (Figure 1c-1d) and stellate cells (Figure 2c-2d) as well as vacuolations observed in the entorhinal cortex (Figure 2d).

Degeneration of pyramidal cells was reported in the hippocampus of adult wistar rats directly exposed to MCS for 1 to 3 hours daily for 28 days (2). Another study has shown that dermal application of NN-diethyl m toluamide and permethrin at subchronic levels caused diffuse neuronal cell loss in the hippocampus, cerebral cortex and cerebellum in adult rats (17). Moreover, decreased levels of brain antioxidant; superoxide dismutase, reduced glutathione and catalase was reported in the offsprings following gestational exposure to bifenthrin and cyfluthrin-based pyrethroids (11), and allethrin-based liquid mosquito repellant (8).

Mosquito coil smoke contain many gases and particles like carbon monoxide (CO), carbon dioxide (CO2), Pyro(a)benzene and other hydrocarbon(18). CO is known for its ability to supplant hemoglobin of its oxygen and thereby, exposing the neuronal cells to hypoxic state (19). This may result in arrest of cellular functions and leads to cellular death (18). Mosquito coil smoke also releases gases and particles that could interfere with the normal cellular processes of energy generation (20). One of the first results of energy depletion may involve the failure of Na+ and K+ pumps with associated depolarized state of the neuron membrane. This is further associated with failure of synaptic function and conductivity (21). Glutamate is released into the synaptic cleft as a

result of neuronal depolarization leading to the excitation of neurons. But when the glutamate is in excess, it becomes an excitotoxin (21).

Since some glutamate receptors are non-selective cation-permeable ion channels, over activation of these channels causes a passive influx of Cl- and Na+ into cells causing cytotoxic oedema and rapid cell death (21). Additional structural damage develops hours or days as a result of Ca+ influx into neurons (21). These alterations could be the reasons for the neuronal degeneration and other structural changes observed in this study.

CONCLUSION

This study has shown that exposure to mosquito coil smoke during gestation causes alteration in the histomorphology of the hippocampus and entorhinal cortex of prenatally exposed adolescent Wistar rats and could increase the risk for memory impairment.

RECOMMENDATION

Further studies should be carried out on neurochemistry to ascertain the level of antioxidants depletion in relation to prenatal exposure to mosquito coil smoke. Also, Pyrethroid-based mosquito coil users should employ safety measures when using the product.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Sinha C, Agrawal AK, Islam F, Seth K, Chaturvedi RK, Shukla S et al. Mosquito repellent (pyrethroid-based) induced dysfunction of bloodbrain barrier permeability in developing brain. *Int. J. Dev. Neurosci 2004 ;* 22 : 3137
2. Mahmood U, Adebayo B, Ahmed SI, Abubakar SA, Muhammad MM, Haruna DU. Effect of Exposure to Mosquito Coil Smoke on Short-Term Memory of Adult Wistar Rats. *Nig. J. Neurosci. 2018 ; 9(1) : 24-28*
3. Ibrahim KR, Hala ZE. Histological changes of the adult albino rats' entorhinal cortex under the effect of tramadol administration: Histological and Morphometric study *Alexandria J. Med.* 2016 ; 53 : 123 133
4. Imanishi S, Okura M, Zaha H, Yamamoto T, Nagano R, Shiraishi H, Sone H. Prenatal exposure to permethrin influences vascular development of fetal brain and adult behavior in mice offspring. *Environ Toxicol.* 2013 ; 28(11): 617- 629
5. Buzsaki G, Moser EI. Memory navigation and theta rhythm in the hippo campal- entorhinal system. *Nat. Neurosci. 2013* ; 16 : 130138
6. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. *Nature 2005 ;* 436 : 801806
7. Solstad T, Boccara CN, Kropff E, Moser MB, Moser EI. Representation of geometric borders in the entorhinal cortex. *Science.* 2008 ; 322 : 18651868
8. Sinha C, Seth PK, Islam F, Chaturvedi RK, Shukla S, Mathur N, Srivastava N, Agrawal AK. Behavioral and neurochemical effects induced by pyrethroid-based mosquito repellent exposure in rat offsprings during prenatal and early postnatal period. *Neurotoxicol Teratol.* 2006 ; 88 : 472481
9. Yang Y, Zhou J, Liu J, Liu W. Joint toxicity of permethrin and cypermethrin at sublethal concentrations to the embryo-larval zebrafish. *Chemosphere* 2014; 96 : 146-154
10. David ER, Jeffrey RF. A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol. Ther. 2006* ; 111 : 174 193
11. Syed F, John P, Inderpal S. Neurodevelopmental Consequences of Gestational and Lactational Exposure to Pyrethroids in Rats. *Environ. Toxicol. 2015;* 12: 1761- 1770
12. Khan MK, Asmatullah. Teratogenic Effect of Sublethal Doses of Deltamethrin in Mice. *Pakistan J. Zool.* 2013 ; 45(3) : 857-864
13. Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ Health Perspect.* 2005 ; 113 : 123 136
14. Andleeb S. Teratogenic potential of pyrethroids: a review *Punjab Univ. J. Zool. 2016* ; 31(1) : 107-125
15. Hamid HY, Zakaria A. Reproductive characteristics of the female laboratory rat; A Review. *Academic Journals* 2012 ; 2(19) : 2510-2514
16. Mshelia PP, Magaji RA, Dikko AU. Cognitive Effect of Sub-Acute Exposure to Mosquito Coil Smoke in Mice. *J Pharm Bioll Sci.* 2013 ; 8(2) : 26-30
17. Abdel-Rahman A, Shetty AK, Abou-Donia MB. Subchronic dermal application of NN-diethyl m-toluamide (DEET) and permethrin to adult rats alone or in combination causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus and Purkinje neuron loss in the cerebellum.*Experimental Neurology.* 2001 ; 172 : 153171
18. Abubakar M, Hassan L. Toxicological Effects of Some Mosquito Coils Brands in Experimental Rats. *The Internet Journal of Toxicology*. 2006 ; 4(1) : 45-49
19. John K, Bryan H, Amanda F, Jeffrey KW, Maureen YL, William BH, David K. Analysis of Pesticides and Toxic Heavy Metals Contained in Mosquito Coils. *Bull Environ Contam Toxicol.* 2016 ; 97 : 614-618
20. Liu W, Zhang J, Hashim JH, Jalaludin J, Hashim Z, Goldstein BD. Mosquito Coil Emissions and Health. Implications *Environ Health Perspect.* 2003 ; 111 (12) : 14541460
21. Dimitri P, Agamanolis MD. HypoxicIschemic Encephalopathy. *Neuropathology. 2013 ;* 8 : 26-38
22. Tina S, Lisbeth BM, Torben M. Impairment of Interrelated Iron and Copper Homeostatic Mechanisms in Brain Contributes to the Pathogenesis of Neurodegenerative Disorders. *Front. Pharmacol.* 2012 ; 3 : 169-181
23. Akihiko N, George P, Miguel A, Pappolla MA, Robert P, Keisuke H. Neuronal Oxidative Stress Precedes Amyloid-b Deposition in Down Syndrome. *J. Neuropath. Exp. Neurol.* 2000 ; 59(11) : 1011-1017
24. Pappolla MA, Omar RA, Kim KS, Robakis NK. Immunohistochemical evidence of oxidative stress in Alzheimer's disease *Am. J. Pathol.* 1992; 140: 62128
25. Hardy J. Amyloid the presenilins and Alzheimer's disease. Trends Neurosci. 1997 ; 20 : 15459