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Histopathological Impact of Gasoline Vapor Inhalation on the Olfactory Epithelium of Wistar Rats

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ABSTRACT

A common route of exposure to gasoline is the olfactory epithelium. The aim of this study was to investigate the effect of gasoline vapor on the histology of the olfactory epithelium and on some biomarkers of oxidative stress. The experiment lasted for 21 days with 35 rats designated into 7 groups. Rats exposed to gasoline were placed in whole body exposure chambers with 500mls of gasoline. The rats were exposed for between 5-30minutes depending on the group for exposure. The histopathology of the olfactory epithelium revealed loss of sustentacular cells (5, 10, 15, 20-minute exposure), proliferation of basal cells (5, 10, 15, 20, 25 minutes), thinning of the olfactory epithelium (10-minute exposure), loss of the apical surface of the olfactory epithelium (15 and 20-minute exposure)and apoptosis (30-minute exposure). Increase in malondialdehyde ($p<0.05$) was observed after exposure to gasoline (5, 10, 15, 20, 25, 30-minutes). A decrease in superoxide dismutase ($p>0.05$) (5, 10, 20, 25, 30-minutes) and a decrease in catalase ($p< 0.05$) (5, 10, 15, 20, 25, 30-minutes) was also observed. These results indicate that exposure to gasoline altered the normal histological architecture of olfactory epithelium and induced oxidative stress in albino Wistar rats.

Keywords: Gasoline, Olfactory, Epithelium, Histopathology, Vapor

INTRODUCTION

The olfactory epithelium is the organ responsible for the sense of smell which is located in the nasal cavity [1]. It is located at the superior medial vertical lamellae of the superior turbinate's and the corresponding nasal septum [2]. Within the olfactory epithelium are found the olfactory neurons, basal cells and support cells [3]. The support cells also known as sustentacular cells are non-neuronal cells. They constitute a line of cells that inhabits the epithelial full height [4]. The olfactory neuron also known as the olfactory sensory neuron is a neuron that has one axon and one dendrite [5]. Basal cells are regarded as stem cells and have been suggested to regulate neurogenesis [6-7].

Exposure of the olfactory epithelium to the external environment results in the destruction of the olfactory epithelium due to the presence of airborne contaminants [8]. Gasoline is one such contaminant which contains volatile components and the major route of exposure to it is by inhalation[9-10]. The olfactory system is a highly sensitive detector of these volatile chemicals [11].Some of the toxic substances found in gasoline include benzene, toluene, xylene etc.[12]

The health hazards related with chronic or sub-chronic exposure to the pollutants in gasoline has attracted the attention of the general public and the scientific community. Studies on human and experimental animals such as genotoxicity[13], immunotoxicity [14], reproductive toxicity [15], developmental toxicity [16], hematotoxicity, renal function [17], hepatotoxic[18], cancer[19], nephrotoxic [20], lungs [21]etc. have been carried out on gasoline. This study was conducted to determine the effect of gasoline via inhalation on the olfactory epithelium.

MATERIALS AND METHODS

Experimental Procedure and Chemicals: The care of the animals was done according to the animal ethics committee guidelines of the University of Calabar (034ANA3419). Gasoline was purchased from the NNPC filling station at Elele, Rivers State Nigeria. Thirty-five male albino Wistar rats weighing 180-240g were obtained from the animal house of the Department of Anatomy, University of Port Harcourt, Rivers State, Nigeria. The rats were divided into 7 groups designated 5-30 min exposure groups, consisting of 5 rats each. The rats were housed in cleaned cages and maintained at room temperature (23 ± 2◦c) and a 12hr light/dark cycle. The animals were allowed to acclimatize for one week and were given access to food and water *ad libitum*.

Experimental Design: The control group were not exposed to gasoline vapor while the exposure groups were exposed to gasoline vapor for 21 days [22-23]. The exposure groups were as follows: 5-minute (min) exposure group, 10 min exposure group, 15 min exposure group, 20 min exposure group, 25 min exposure group and 30 min exposure group. The groups were exposed to gasoline vapor in exposure chambers.

Exposure to Gasoline Vapor: The method of exposure earlier described [24-25] was adopted for this study. Animals were placed in whole body exposure chambers measuring 23.5 x 17.0 x 18.5 inches. 500mls of gasoline was placed in the chambers 1 hour before the exposure commenced. Animals in all the exposure groups were later placed in the chambers and allowed to inhale the vapour for the duration of each exposure. Rats were removed from the exposure chambers and placed in a vapour free section of the animal house after each exposure. A portable air quality monitor (S-500) was used to

measure the amount of gasoline vapour inhaled by each group of rats.

Collection of Blood/Tissue Samples: At the end of the exposure duration of 21 days, each group of rats were anaesthetized and killed. A 5 ml syringe was used to collect blood samples from the rats by cardiac puncture. The blood was collected from the left ventricle. Blood samples were put in plain bottles so as to obtain the serum. Sera obtained after centrifugation was used for the estimation of oxidative. The rats were decapitated and the entire nasal tissues were obtained from the tip to the ending connexion with the olfactory bulb. The olfactory epithelium was removed and separated from the respiratory epithelium. It was fixed by immersion in 4% paraformaldehyde in phosphate buffer saline.

Statistical Analysis: Data was expressed as Mean ± SEM (standard error of the mean) and one-way Analysis of Variance was utilized to analyze experimental data. Least significant difference multiple tests were utilized to compare between the group mean and the control. Differences were considered significant at P< 0.05.

RESULTS

Readings for Gasoline Vapour Evaporated During Exposure: The concentration of gasoline each group of rats were exposed to was recorded in parts per million (ppm). Each of the groups had the following readings: 5 min exposure (16,737ppm), 10 min exposure (20,240 ppm), 15 min exposure (23,077ppm), 20 min exposure (27,344ppm), 25 min exposure (30,920ppm) and 30 min exposure (34,458 ppm).

Assessment of Oxidative Stress Markers: There was no significant decrease in serum SOD levels (P>0.05) in the exposure groups compared to the control. Fig. 1. There was significant increase in MDA levels (P<0.05) in the exposure groups Fig. 2.while there was significant decrease in CAT levels (P<0.05) in the exposure groups. Fig 3.

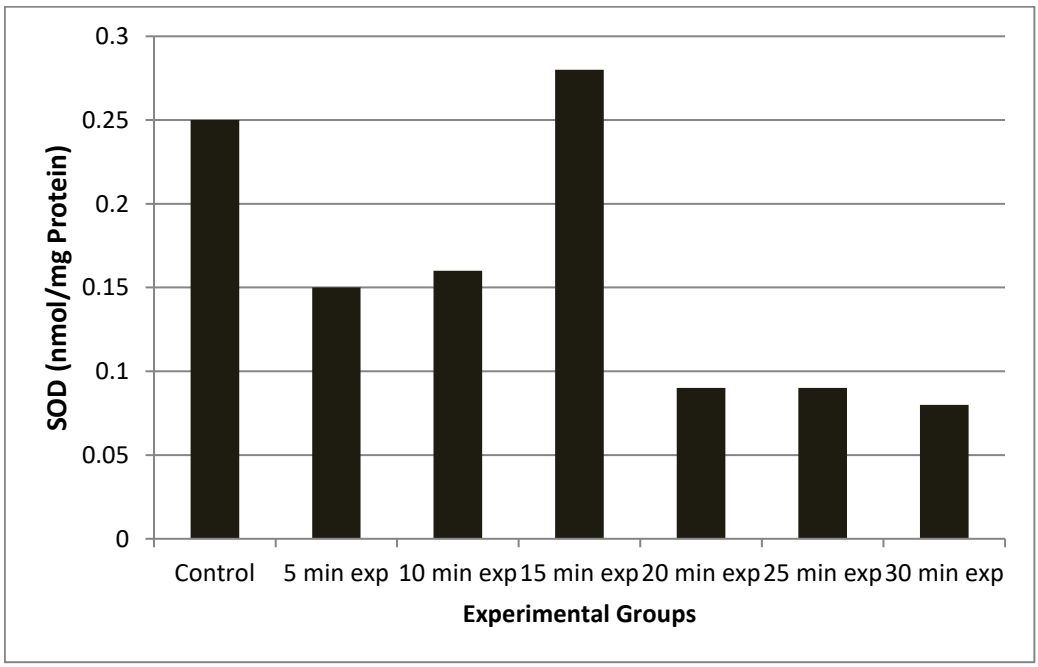

Figure 1: Thelevels of superoxide dismutase (SOD) (p>0.05)

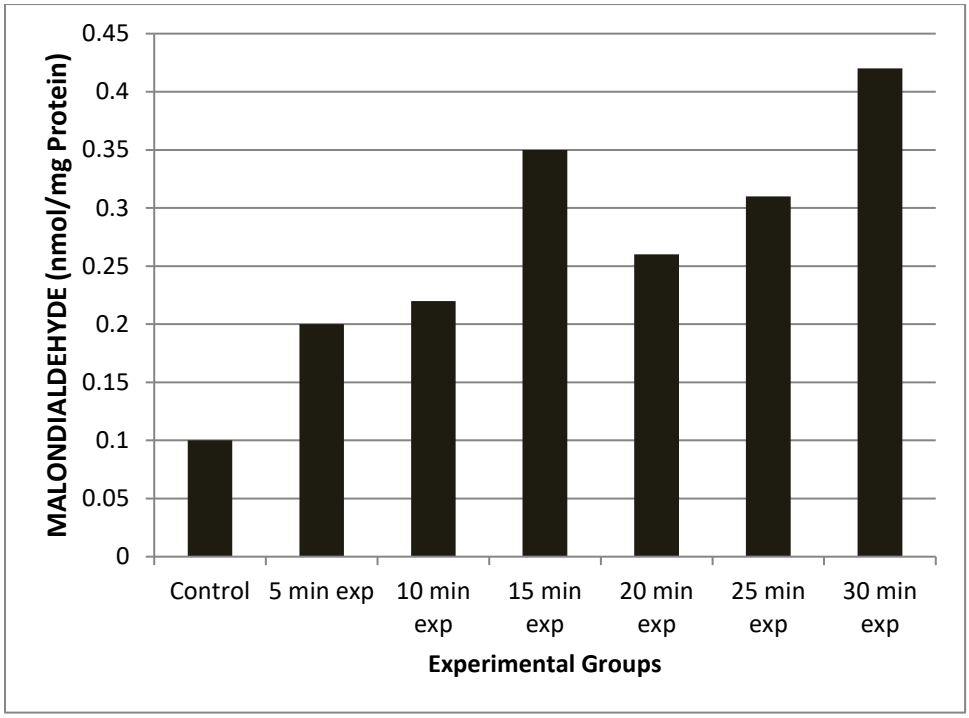

Figure 2: The levels of malondialdehyde (MDA) ($p<0.05$)

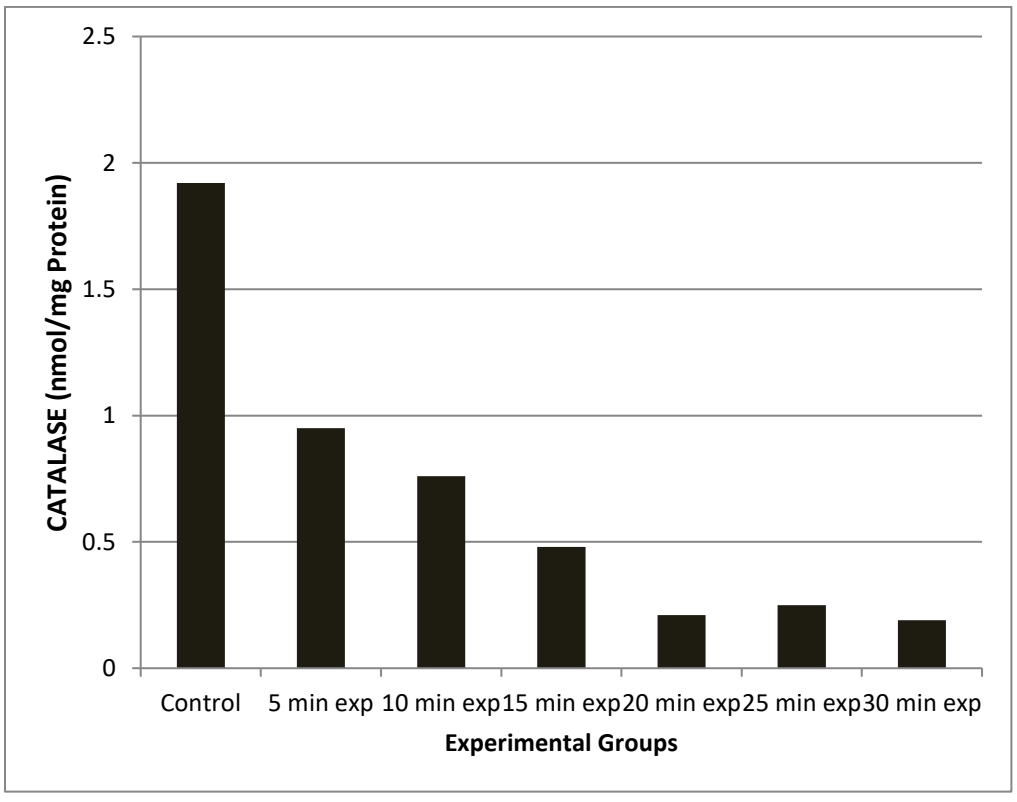

Figure 3: The levels of catalase (CAT) ($p<0.05$)

Nasal Epithelium Histopathology: The olfactory epithelium of the control group was observed to have a normal epithelial lining as shown in fig 4. Following exposure to gasoline for 5minutes; there was a loss of sustentacular cells as well as proliferation of basal cells in the olfactory epithelium shown in fig 5. Loss of sustentacular cells, proliferation of basal cells and thinning of the olfactory epithelium was observed after exposure for 10 minutes shown in fig 6. After exposure for 15 minutes there was a loss of the apical surface of the olfactory epithelium as well as loss of the sustentacular cells and proliferation of basal cells shown in fig 7. Exposure for 20 minutes resulted in loss of sustentacular cells, proliferation of basal cells and further loss of the apical surface of the olfactory epithelium shown in fig8. After exposure for 25 minutes proliferation of the basal cells and total loss of the apical surface of the olfactory epithelium was observed as shown in fig 9. 30 minutes exposure, presented with loss of the olfactory epithelium and apoptosis shown in fig 10.

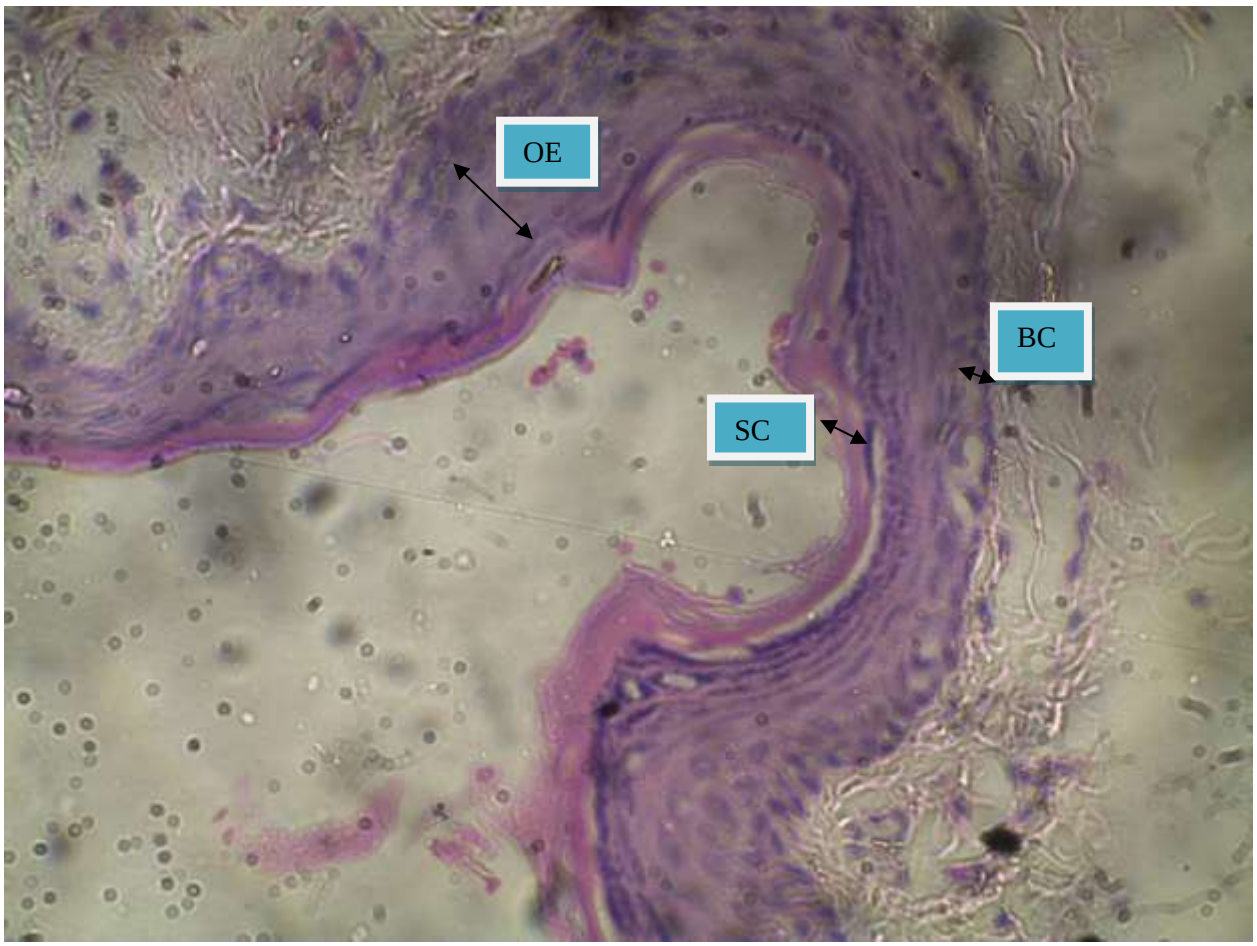

Figure 4: Normal epithelial lining (OE), Sustentacular cell (SC), Basal cell (BC) (H&E x500) (control group)

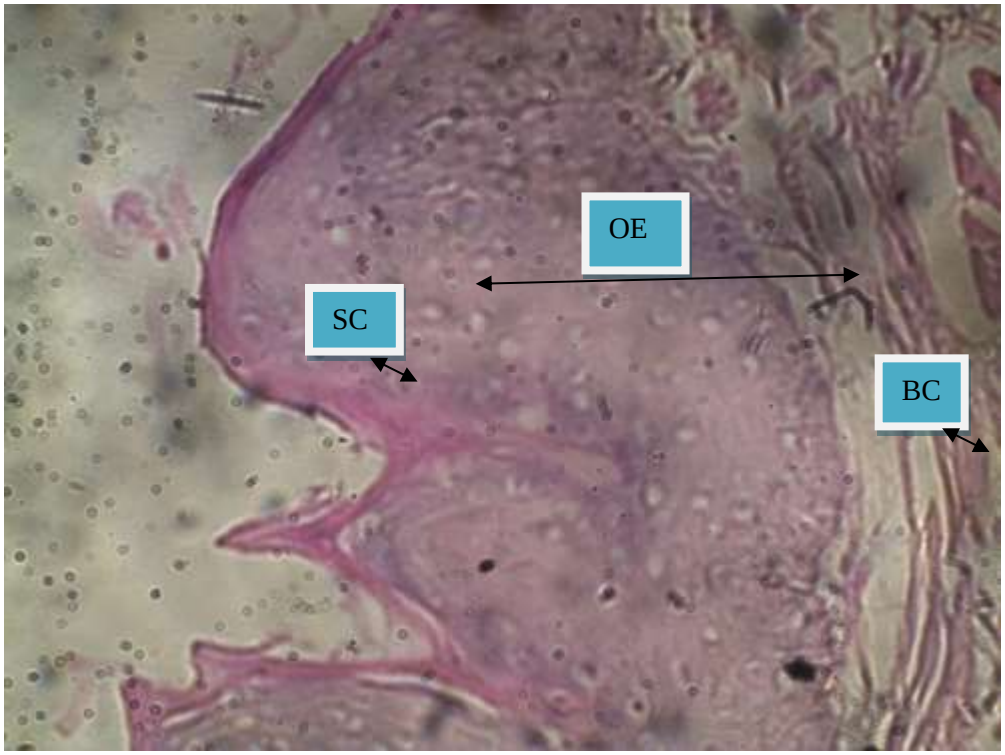

Figure 5: Loss of Sustentacular cells (SC) and proliferation of basal cells (BC), Olfactory epithelium (OE). (H&E x500) (5 min exposure)

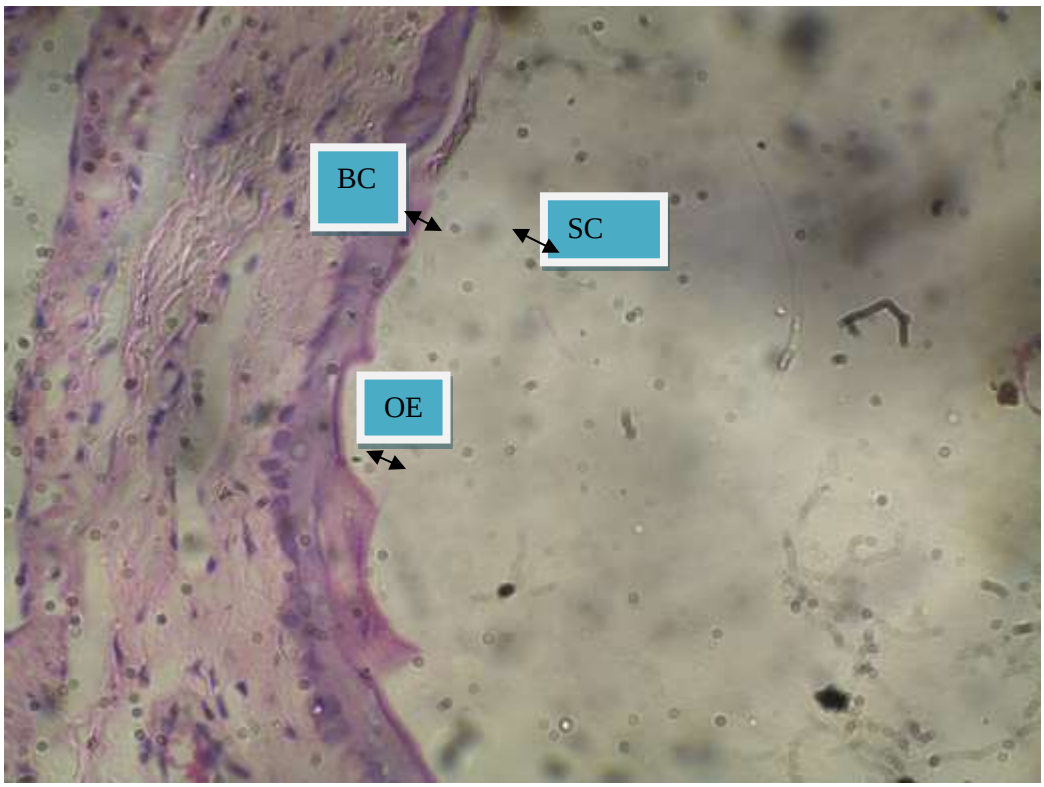

Figure 6: Loss of sustentacular cells (SC), proliferation basal cells (BC) and thinning of the olfactory epithelium OE. (H&E × 500) (10 min exposure)

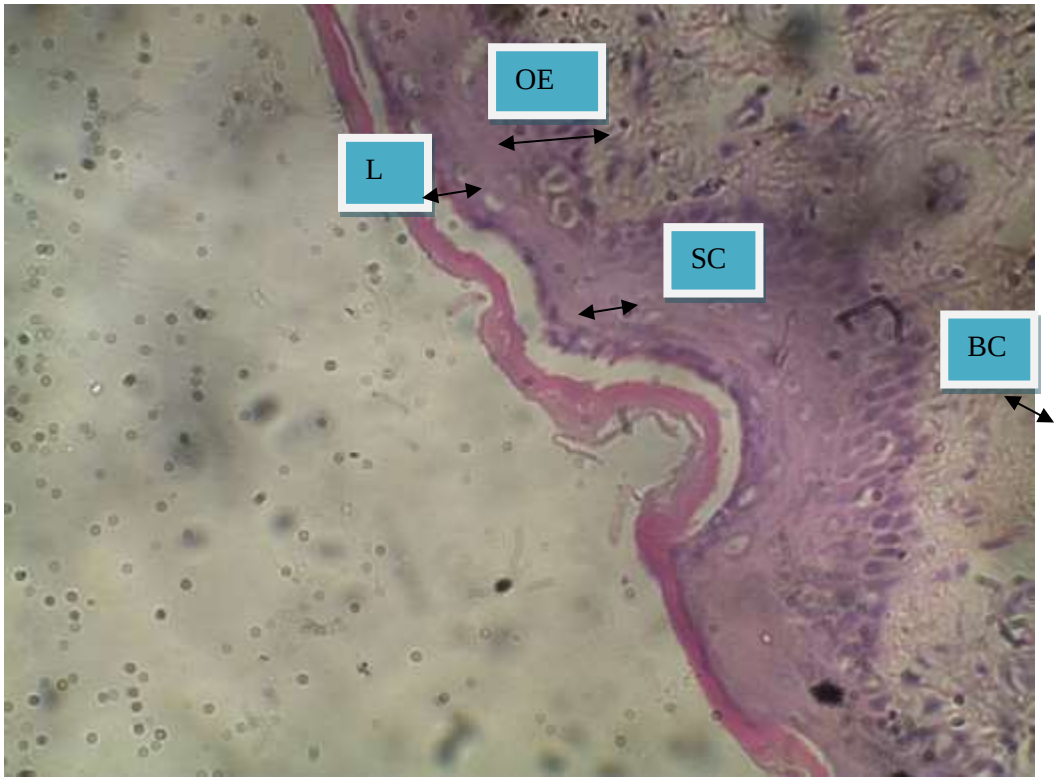

Figure 7: Loss (L) of apical surface of olfactory epithelium (OE), loss of sustentacular cells (SC) and proliferation of basal cells (BC) (H&E × 500) (15 min exposure)

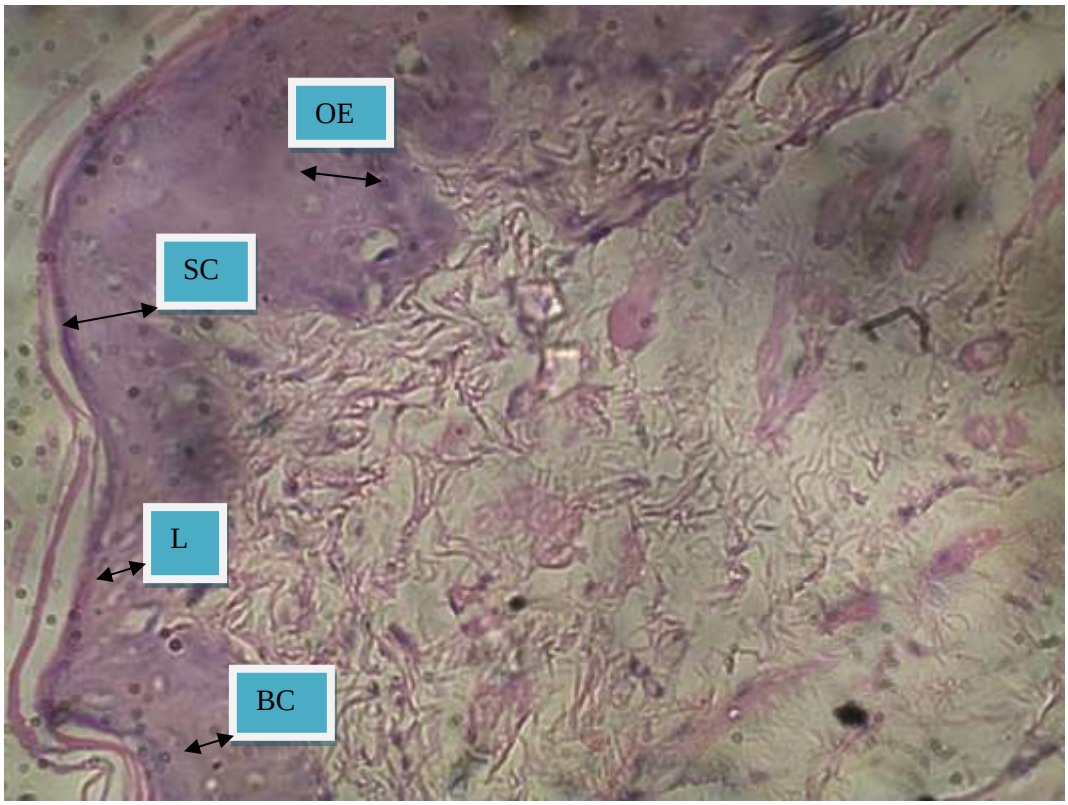

Figure 8: Loss of sustentacular cells (SC), proliferation of basal cells(BC) and further loss (L) of the apical surface of the olfactory epithelium(OE) (H&E × 500) (20 min exposure)

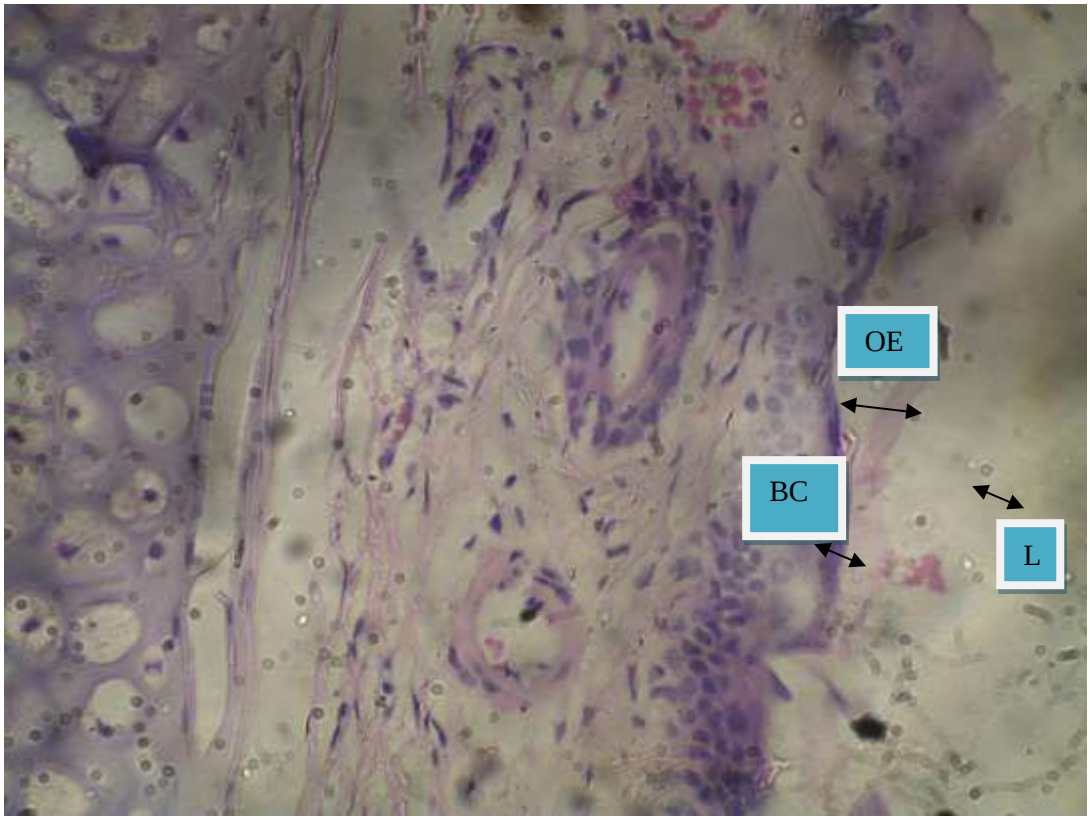

Figure 9: Proliferation of basal cells (BC), total loss of apical surface of olfactory epithelium (L) (H&E × 500) (25 min exposure)

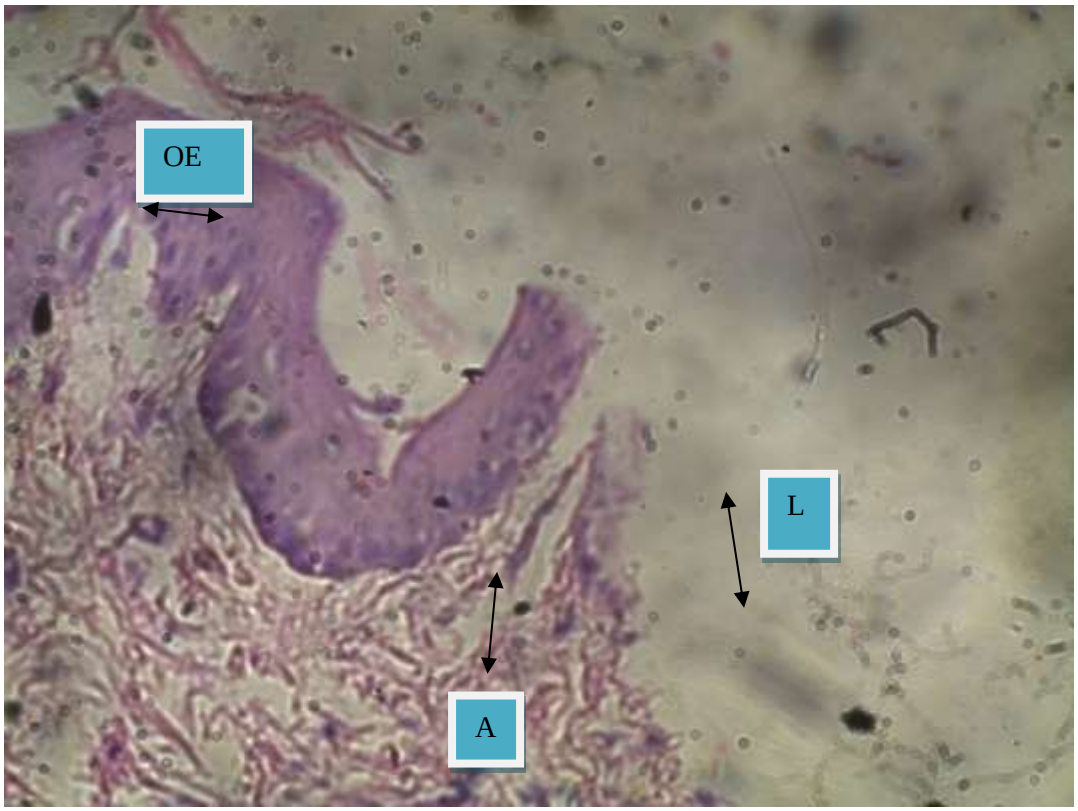

Figure 10: Loss of olfactory epithelium (L), apoptosis (A) (H&E × 500) (30 min exposure)

DISCUSSION

The present study was carried out to evaluate the effect of gasoline vapour inhalation on the levels of oxidative stress markers (MDA, SOD and CAT) and the olfactory epithelium. Gasoline is found abundantly in the atmosphere as a result of its volatile nature hence lots of Nigerians are exposed to it [26-27]. A common channel of exposure to the toxic substances found in gasoline is by inhalation due to its high vapour pressure [9-10].

In this study the histopathological examination of the olfactory epithelium revealed loss of sustentacular cells, proliferation of basal cells, loss of apical surface of the olfactory epithelium and cellular apoptosis. It was stated [28]that nasal tissue contact with chemicals during breathing may affect the longevity of the olfactory epithelium cells. This was observed in this study in which as the olfactory epithelium was exposed to gasoline, there was loss of sustentacular cells. In another study[29] it was also reported that there was loss of sustentacular cells after exposure of the olfactory epithelium to nickel. The progression from cell loss to apoptosis in this study can be linked to another work[30—31]in which it was reported that the outcome of the olfactory mucosa after contact with a pollutant is dependent on the duration of the contact and the concentration of the pollutant. In this study the cells in the olfactory epithelium progressed from cellular loss to cellular apoptosis which could be explained as occurring due to increase in the duration of exposure. It was reported [32]that elevation in epithelial proliferation is a true indicator of toxic chemicals in the air. This report could explain the proliferation of basal cells which occurred after exposure to gasoline in this study. Despite the position of the olfactory epithelium from the main airstream, xenobiotics still readily get to this region [33]. The sustentacular cells have been reported to have a tremendous capacity for xenobiotic metabolism[34]. The findings from the histopathological examination of the olfactory epithelium in this study can be stated to be as a result of the interaction of gasoline with the xenobiotic metabolizing enzymes in the sustentacular cells and the bowman's glands.

One of the terminal end products of polyunsaturated fatty acids peroxidation in cells is MDA which is a marker of oxidative stress [35]. Lipid peroxidation helps in the assessment of deterioration brought about by free radicals on membrane lipids [36]. In this study a significant increase in the level of MDA was observed in all the groups exposed to gasoline. This could explain the deterioration in the state of the sustentacular cells observed in this study. A study [37] reported a significant increase in lipid peroxidation after rats were exposed to leaded gasoline for 30mins/ day for 6 weeks. Another study [38] reported an increase in MDA and a decrease in SOD and CAT in rats after exposure to gasoline 5 mins/day for 8 weeks. These reports back the findings in this study and can further suggest that short term exposure to gasoline results in the occurrence of lipid peroxidation. The net result of oxidative stress is damage to the cellular frame work, function of the enzyme and stability of the genome[39]. Results from a study [21]stated a decline in SOD levels after exposure to gasoline for 30 mins/day for six weeks. It was reported [18]that there was an increase in MDA and a decrease in SOD and CAT among filling station workers. The results of this present study indicated that exposure of rats to gasoline for 5-30 mins/day for 21 days resulted in oxidative stress as seen in the increase in MDA and the decrease in the SOD and CAT levels.

CONCLUSION

The results from this study indicated that gasoline exposure could bring about alteration in the histoarchitecture of the

olfactory epithelium as well as result in oxidative stress in Wistar rats.

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CONFLICT OF INTEREST

None declared

REFERENCES

1. Choi R, Goldstein BJ. Olfactory epithelium: Cells, clinical disorders, and insights from an adult stem cell niche. Laryngoscope investigative otolaryngology. 2018; 3(1):35-42.
2. Holbrook EH, Wu E, Curry WT, Lin DT, Schwob JE. Immunohistochemical characterization of human olfactory tissue. The Laryngoscope. 2011; 121(8):1687-701.
3. Soudry Y, Lemogne C, Malinvaud D, Consoli SM, Bonfils P. Olfactory system and emotion: common substrates. European annals of otorhinolaryngology, head and neck diseases. 2011; 128(1):18-23.
4. Vedin V. *Molecular and functional anatomy of the mouse olfactory epithelium* (Doctoral dissertation, Molekylärbiologi).
5. Mendoza AS. Morphological studies on the rodent main and accessory olfactory systems: the regio olfactoria and vomeronasal organ. Annals of Anatomy-Anatomischer Anzeiger. 1993; 175(5):425-46.
6. Brescia AI, Seiden AM. The anatomy and physiology of olfaction and gustation. InRhinology and Facial Plastic Surgery 2009 (pp. 91-96). Springer, Berlin, Heidelberg.
7. Plopper C, Harkema JR. The respiratory system and its use in research. The laboratory primate 2005; 503-526
8. Calderon-Garciduenas L, Azzarelli B, Acuna H, Garcia R, Gambling TM, Osnaya N, Monroy S, Del Rosario Tizapantzi M, Carson JL, Villarreal-Calderon A, Rewcastle B. Air pollution and brain damage. Toxicologic pathology. 2002; 30(3):373-89.
9. Carballo M, Nigro ML, Fraga I, Gadano A. Ethylene oxide: cytogenetic and biochemical studies in persons occupationally exposed. Environ Mol Mutagen. 1994; 23(23):7-12.
10. Cecil R, Ellison RJ, Larnimaa K, Margary SA, Mata JM, Morcillo L, Muller JM, Peterson DR, Short D, Simpson BJ. Exposure profile: gasoline. REPORT-CONCAWE-WHITE SPECIAL INTEREST-. 1997.
11. Hudson R. From molecule to mind: the role of experience in shaping olfactory function. Journal of Comparative Physiology A. 1999; 185(4):297-304.
12. Owagboriaye FO, Dedeke GA, Aladesida AA, Bamidele JA, Olooto WE. Assessment of the effect of gasoline fume on stress hormones, antioxidant status and lipid peroxidation in albino rat. Journal of king Saud university-science. 2018; 30(3):393-9.
13. Schreiner CA, Hoffman GM, Gudi R, Clark CR. Health assessment of gasoline and fuel oxygenate vapors: micronucleus and sister chromatid exchange evaluations. Regulatory Toxicology and Pharmacology. 2014; 70(2):S29-34.
14. White Jr KL, Peachee VL, Armstrong SR, Twerdok LE, Clark CR, Schreiner CA. Health assessment of gasoline and fuel oxygenate vapors: immunotoxicity evaluation. Regulatory Toxicology and Pharmacology. 2014; 70(2):S43-7.
15. Gray TM, Steup D, Roberts LG, O'Callaghan JP, Hoffman G, Schreiner CA, Clark CR. Health assessment of gasoline and fuel oxygenate vapors: reproductive toxicity assessment. Regulatory Toxicology and Pharmacology. 2014; 70(2): S48-57.

16. Roberts LG, Gray TM, Marr MC, Tyl RW, Trimmer GW, Hoffman GM, Murray FJ, Clark CR, Schreiner CA. Health assessment of gasoline and fuel oxygenate vapors: developmental toxicity in mice. Regulatory Toxicology and Pharmacology. 2014 ; 70(2):S58-68.
17. Uboh FE, Akpanabiatu MI, Eteng MU, Ebong PE, Umoh IB. Toxicological effects of exposure to gasoline vapor in male and female rats. Internet J Toxicol. 2008;4(2):1-6.
18. Odewabi AO, Ogundahunsi OA, Oyalowo M. Effect of exposure to petroleum fumes on plasma antioxidant defense system in petrol attendants. British Journal of Pharmacology and Toxicology. 2014; 5(2):83-8.
19. Lynge E, Andersen A, Nilsson R, Barlow L, Pukkala E, Nordlinder R, Boffetta P, Grandjean P, Heikkiia P, Horte LG, Jakobsson R. Risk of cancer and exposure to gasoline vapors. American journal of epidemiology. 1997; 145(5):449-58.
20. Uboh FE, Akpanabiatu MI, Ndem JI, Alozie Y, Ebong PE. Comparative nephrotoxic effect associated with exposure to diesel and gasoline vapors in rats. Journal of Toxicology and Environmental Health Sciences. 2009; 1(4):068-74.
21. Ezzat AR, Riad NH, Fares NH, Hegazy HG, Alrefadi MA. Gasoline inhalation induces perturbation in the rat lung antioxidant defense system and tissue structure. IJESE. 2011;1:1-4.
22. Okonkwo CO, Ehileboh AD, Nwobodo E, Dike CC. The effects of acute gasoline vapor inhalation on some hematological indices of albino Wistar rats. Journal of acute disease. 2016; 5(2):123-5.
23. Elsayed AS, Hegazi MA. Ameliorative Effect of Curcumin and Green Tea against Gasoline Inhalation Hematotoxicity. Int. J. Appl. Biol. Pharm. Technol. 2016;7(1).
24. Uboh FE. Evaluation of toxicological implications of inhalation exposure to kerosene fumes and petrol fumes in rats. Acta Biologica Szegediensis. 2005; 49(3-4):19-22.
25. Uboh FE, Akpanabiatu MI, Atangwho IJ, Ebong PE, Umoh IB. Effect of gasoline vapors on serum lipid profile and oxidative stress in hepatocytes of male and female rats. Acta Toxicologica. 2007; 15(1).
26. Okoro AM, Ani EJ, Ibu JO, Akpogomeh BA. Effect of petroleum products inhalation on some hematological indices of fuel attendants in Calabar metropolis, Nigeria. Nigerian Journal of Physiological Sciences. 2006; 21(1-2).
27. Ita SO, Udofia UA. Comparative study of some hematological parameters in rats following ingestion of crude oil (Nigerian Bonny Light), petrol, kerosene and diesel. Asian J. Biol. Sci. 2011; 4(6):498-505.
28. Calderón-Garcidueñas L, Rodríguez-Alcaraz A, Villarreal-Calderón A, Lyght O, Janszen D, Morgan KT. Nasal epithelium as a sentinel for airborne environmental pollution. Toxicological Sciences. 1998; 46(2):352-64.
29. Evans JE, Miller ML, Andringa A, Hastings L. Behavioral, histological, and neurochemical effects of nickel (II) on the rat olfactory system. Toxicology and applied pharmacology. 1995; 130(2):209-20.
30. Newton PE, Bolte HF, Derelanko MJ, Hardisty JF, Rinehart WE. An evaluation of changes and recovery in the olfactory epithelium in mice after inhalation exposure to methylethylketoxime. Inhalation toxicology. 2002; 14(12):1249-60.
31. Katagiri T, Takeuchi T, Mine T, Noguchi T, Nishizawa T, Yamamoto S, Okudaira M, Matsushima T. Chronic inhalation toxicity and carcinogenicity studies of 3-chloro-2-methylpropene in BDF1 mice. Industrial health. 2000; 38(3):309-18.
32. Villalobos-Pietrini R, Amador-Muñoz O, Waliszewski S, Hernández-Mena L, Munive-Colín Z, Gómez-Arroyo S,

Bravo-Cabrera JL, Frías-Villegas A. Mutagenicity and polycyclic aromatic hydrocarbons associated with extractable organic matter from airborne particles⩽ 10 μm in southwest Mexico City. Atmospheric Environment. 2006; 40(30):5845-57.
33. Doty RL, Hastings L. Neurotoxic exposure and olfactory impairment. Clin Occup Environ Med. 2001; 1:547-75.
34. Dahl AR, Hadley WM. Nasal cavity enzymes involved in xenobiotic metabolism: effects on the toxicity of inhalants. Critical reviews in toxicology. 1991; 21(5):345-72.
35. Gaweł S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2004; 57(9-10):453-5.
36. Baydas G, Ozveren F, Tuzcu M, Yasar A. Effects of thinner exposure on the expression pattern of neural cell adhesion molecules, level of lipid peroxidation in the brain and cognitive function in rats. European journal of pharmacology. 2005; 512(2-3):181-7.
37. Kinawy AA. Impact of gasoline inhalation on some neurobehavioural characteristics of male rats. BMC physiology. 2009; 9(1):1-0
38. Azeez OM, Akhigbe RE, Anigbogu CN. Exposure to petroleum hydrocarbon: implications in lung lipid peroxidation and antioxidant defense system in rat. Toxicology International. 2012; 19(3):306.
39. Gille G, Sigler K. Oxidative stress and living cells. Folia Microbiologica. 1995; 40(2):131-52.