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HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN THE LUNG INDUCED BY SHORT AND LONG TERM EXPOSURE TO XYLENE

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ABSTRACT

Inhalation of xylene is a dangerous type of exposure to this chemical. This study was carried out to investigate the effect of xylene on the histoarchitecture of the lung. Twenty five wistars were used for this work. The rats were randomly divided into five groups A, B, C, D and E of five rats each. Group A served as the control and was not to exposed to xylene, while B, C, D, and E was exposed to xylene for 14 days, 28 days, 42 days and 56 days respectively. The animals had difficulty in breathing, wheezed and coughed. The animals were sacrificed after 14 days, 28 days, 42 days and 56 days and the lung harvested and processed using normal histological technique with Papanicolaou staining and Pararosanilin-Toluidine B staining procedures. The result revealed that xylene fume altered the histoarchitecture of lung and esterase and collagen wall. This ranged from mild emphysematous change, inflammation of cells, Collapse of the collagen wall and distortion of esterase. There was significant increase in weight of the animals in all the groups. In conclusion, xylene fume altered the histology and histochemistry of lung tissues and had no effect on body weight of the animals.

KEYWORDS: Xylene, Lung, Histoarchitecture, Histochemistry, pneumocytes.

INTRODUCTION

Inhalation of xylene is a dangerous type of exposure to this chemical. Xylene is an aromatic hydrocarbon widely used in industry and medical technology as a solvent. It is a colorless, sweet-smelling liquid or gas occurring naturally in petroleum, coal and wood tar, and is so named because it is found in crude wood spirit (Gr. xy`lon- wood) (1). Xylene is used as a solvent in the printing, rubber, paint and leather industries. It is found in small amounts in airplane fuel, gasoline and cigarette smoke. In dentistry, xylene is used in histological laboratories for tissue processing, staining and cover slipping and also in endodontic retreatment as a guttapercha solvent. Its high solvency factor allows maximum displacement of alcohol and renders the tissue transparent, enhancing paraffin infiltration. In staining procedures, its excellent de waxing and clearing capabilities contribute to brilliantly stained slides[1] The common side effect of inhaled xylene is depression of the central nervous system causing dizziness, headache, nausea and vomiting. Irritation of the nose and throat may also occur with low-level inhalation of xylene.[2] Histopathological technicians who routinely come in contact with xylene-contaminated solvents in the workplace are the population most likely to be exposed to high levels of xylene. The current Occupational Safety and Health Administration permissible exposure limit for xylene is 100 ppm as an 8-h time-weighted average (TWA) concentration.[3] Irritation of the nose and throat can occur at approximately 200 ppm after 3–5 min. Accidental splash in the eye may damage the surface of the eye, which will heal within a few days.[4]

MATERIALS AND METHODS

Procurement of xylene: Xylene and other laboratory reagents were purchased and not was purchased from a chemical store in Abakaliki, Ebonyi State.

Animal Procurement

Twenty (20) adult wistar rats of average weight of 120g were purchased from the Animal House of the Department of Anatomy, College of Medicine, Ebonyi State University, Abakiliki. The rats were kept in well ventilated conditions in the animal house of the Department of Anatomy and given normal rat feed and water ad libitum and allow two week period of acclimatization.

Animal Grouping

The animals were randomly divided into five (5) groups: A, B, C, D, and E of five animals. Group A served as the control, while B, C, D and E where the experimental group. while the test groups were exposed to 15mls of xylene each daily at the same time.

Group A animals were not exposed to xylene fumes throughout the period of the experiment.
Group B animals were exposed to 15mL of xylene fumes for 12 hours for 2 weeks (14 days)
Group C animals were exposed to 15mL of xylene fumes for 12 hours for 4 weeks (28 days)
Group D animals were exposed to 15mL of xylene fumes for 12 hours for 6 weeks (42 days)
Group E animals were exposed to 15mL of xylene fumes for 12 hours for 8 weeks (56 days)

Ttreatment schedule

GROUP	TREATMENT	DOSAGE	TIME/DURATION
A (CONTROL)	Nil	Nil	56 days
B	Xylene	15mls	12 hourly for 14 days
C	Xylene	15mls	12 hourly for 28 days
D	Xylene	15mls	12 hourly for 42 days
E	Xylene	15mls	12 hourly for 56 days

BODY WEIGHT

The body weights of the animals were check at the end of each week during the experimental period.

MODE OF EXPOSURE

The xylene fume was placed in an improvised fume chamber of diameter 75cm X 50cm X 30cm hole was bored on the cover of the chamber to allow little ventilation for breathing of animals. The animals were enclosed to xylene fume in the chamber for 12 hours daily during the experimental period. After exposure the rats were returned to their cages.[5]

ANIMAL SACRIFICE

On the last days of exposure to xylene fumes in each experimental group, All rats were anaesthetized by chloroform inhalation, after which a thoracoabdominal incision was made to expose the chest and the lungs removed with forceps. Each lung was fixed in formol-saline..

HISTOLOGICAL PROCESSING

The tissues were processed using histological and histochemical method. Formol-saline-fixed lungs were dehydrated and embedded in paraffin wax. Eight micrometer-thick sections were

cut on a rotary microtome; and sections were stained using Papanicolaou's staining method and Pararosanilin-Toluidine Blue's staining method.

STATISTICAL ANAYLSIS

Results of the body weight was presented as Mean ± SD. Statistical analysis was performed using ANOVA and $P<0.05$ considered statistically significant.

RESULTS

The animal had difficulty in breathing expressed by wheezing, coughing and at times shivering were observed, sluggishness, loss of appetite during the first few days of exposure and after which they ate normal again, they became less active and as a result of their lack of activity.

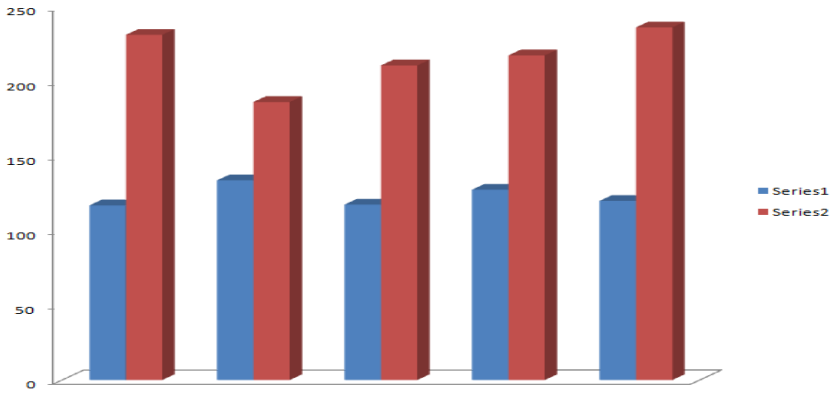
Body weight

The body weights of the animals were check at the end of each week during the experimental period. There was no statistical difference in the body weight of the paint fume exposed groups and the control.

Table 2: Mean value of the initial and final weight of the rats in various groups.

	Mean ±S D	
Groups	Initial weight (g)	Final weight (g)
A	117.00±5.72	231.25±13.15
B	133.75±3.50	186.25±11.09
C	117.50±10.41	210.75±4.35
D	127.50±6.46	217.50±11.90
E	120.00±9.13	236.25±28.69

P<0.05



Groups **A** **B** **C** **D** **E**

Legends

Series 1: initial weight of the animals (g).

Series 2: final weight of the animals (g).

Figure 1. Bar chart showing the initial and final mean weights of the Wistar rats during the experiment.

HISTOLOGICAL AND HISTOCHEMICAL RESULTS

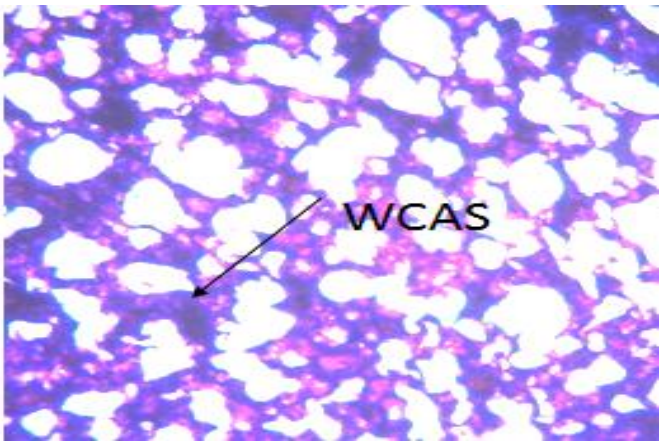


Plate 1b: The photomicrograph of magnification (x100) shows normal lung histoarchitecture of adult Wistar rat represented by well circumscribed alveoli space and normal endothelial cells.

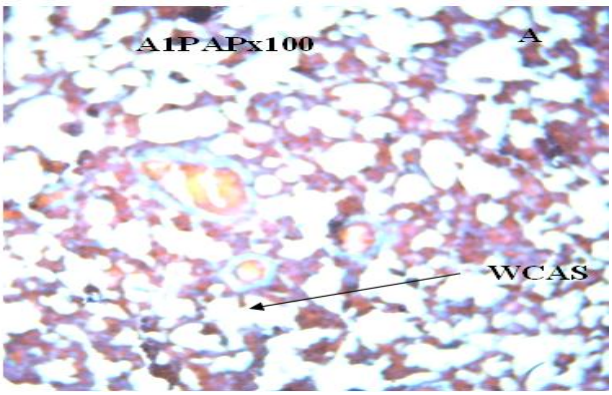


Plate 1a: Thephotomicrograph of magnification x100 shows normal lung histoarchitecture of adult Wistar rat represented by well circumscribed alveoli space (WCAS) and normal endothelial cells (NEC).

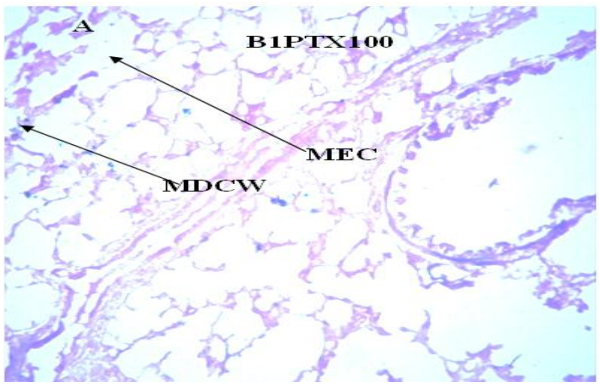


Plate 2b: The photomicrograph of magnification (x100) depicts mild emphysematous change, represented by enlarged alveoli and loss of alveolar septa. It also shows mild distortion of collagen wall in the lung tissues. pararosanilin-Toluidine Blue stains (PT).

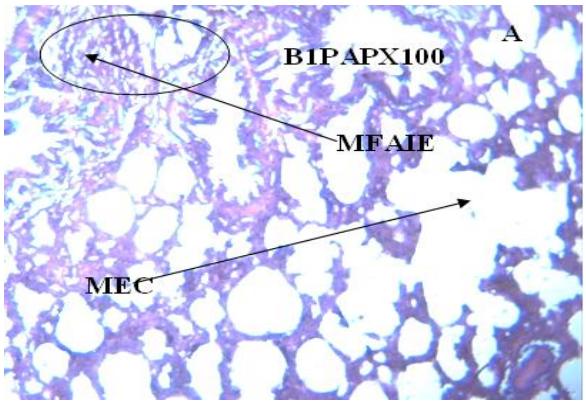
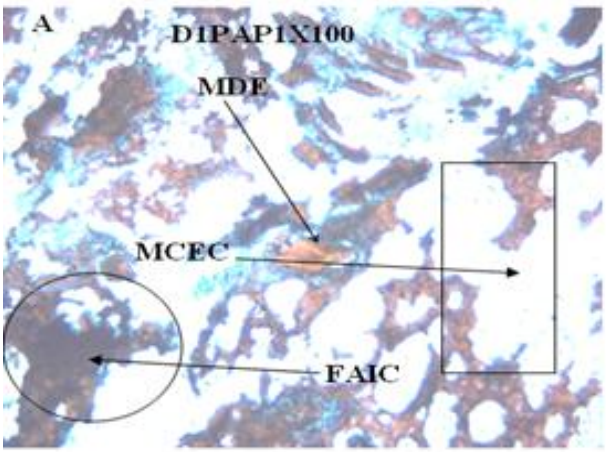


Plate 2a: The photomicrograph of magnification (x100) reveals mild emphysematous change, represented by enlarged alveoli and loss of alveolar septa. It also reveals areas of localized mild inflammation and fluid accumulation in the lung tissue. Stained with papanicolaou's stains.



(Plate 3b): The photomicrograph of Group C magnification(x100) shows mild emphysematous change, represented by enlarged alveoli and loss of alveolar septa in the lung tissues, mild infiltration of inflammatory cell. pararosanilin-Toluidine Blue stains (PT).

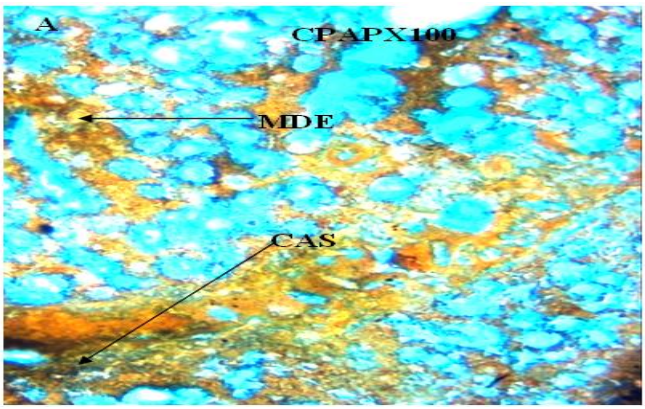


Plate 3a: The photomicrograph of Group C magnification (x100) reveals congestion of the alveolar septa and mild distortion of esterase in the lung, distortion of Type 2 pneumocytes (DT2P). Papanicolaou's staining.

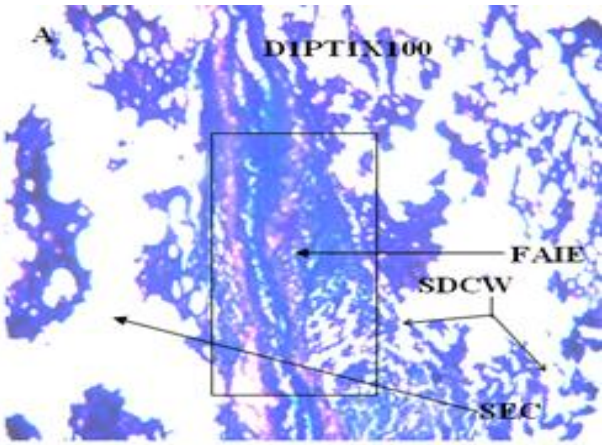


Plate 4b: The photomicrograph Group D of magnification (x100) reveals severe emphysematous change, represented by enlarged alveoli and loss of alveolar septa.focal area of inflammatory exudates distortion of collagen wall (SDCW). Severe distortion of Type 2 pneumocytes (SDT2P) pararosanilin-Toluedine Blue staining.

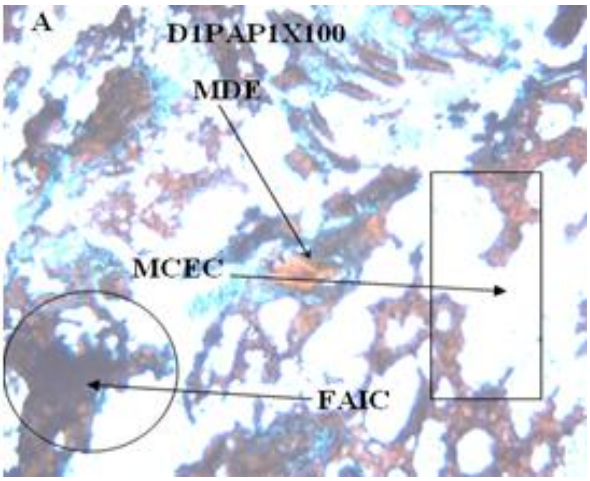


Plate 4a: Photomicrograph of group D magnification x100 reveals moderate effect on the lung with moderate centrobular emphysematous change (MCEC), focal aggregates of inflammatory cells (FAIC), mild distortion of Clara cells (MDCC) and mild distortion of esterase (MDE). papanicolaou's staining.

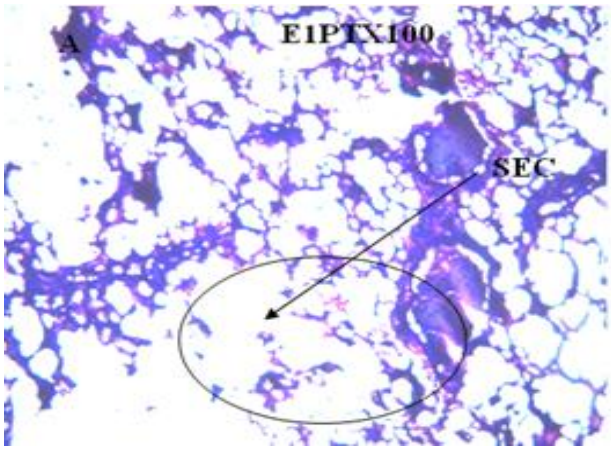


Plate 5b: Photomicrograph of Group E magnification (x100) reveals The severe emphysematous change, represented by enlarged alveoli and loss of alveolar septa in the lung tissues, with severe consolidated focal aggregate of (SCFAIC), pararosanilin-staining.

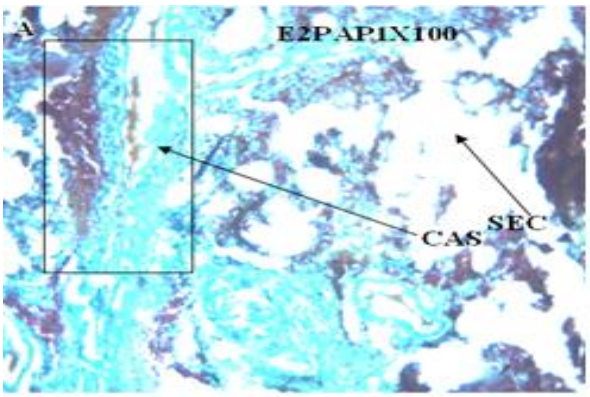


Fig. 5a: Photomicrograph of Group E magnification x100 reveals moderate focal aggregate of inflammatory cells (inflammatory cells MFAIC); severe emphysematous hange (SEC) and congestion of the alveolar septa (CAS) papanicolaou's staining (PAP).

DISCUSSION

Xylene is a hydrocarbon known for its wide usage in tissue processing, staining in the histology laboratory. Exposure occurs primarily by inhalation. The volatility and lipophilicity of the xylenes make the lung and nasal mucosa the primary target organs.[6] Xylene have been reported to cause irritant effect on the respiratory tract and alters its functional activity and cellular structure.[7] The results of this study revealed, that xylene had no significant effect on the body weight of the animals, as the both the weight of the control and the experimental group all increased, almost to the same extent.

The results equally, revealed mild emphysematous change (MEC) to severe emphysematous change (SEC), loss of alveolar septa and mild focal area of inflammatory exudates (MFAIE) and distortion of Type 1 and Type 2 pneumocytes (DT2P); with moderate intra alveoli hemorrhage (MIAH). These alterations increased with the duration of exposure to xylene. The emphysematous change could be responsible for the difficulty in breathing observed in the experimental group after exposure to xylene, and the subsequent sluggishness. These assault to the structural integrity of the lung generally affects the entire lung function and health. The type 11 pneumocytes produce and secrete epithelial lining fluid (ELF) and surfactant.[8] Surfactant allows the alveoli to expand without bursting during inhalation and keeps the walls of the alveoli from sticking together during exhalation.[9] The distortion of these cells will affect surfactant production which could lead to collapse of the lung. Inflammation is a tissue response tissue response to injury or assault, the infiltration of inflammatory cells suggest that xylene has an injurious effect on the epithelium of the lung and so exposure should be controlled. Our findings on the effect of xylene on the alterations of lung histoarchitecture agrees with the findings of Arslan, 2016 in which he reported that xylene alters the cellular structure of the respiratory tract.

Histochemistry of the of the experimental group showed, mild distortion of collagen wall (MDCW) to severe distortion of collagen wall (SDCW) mild distortion of esterase (MDE). These proteins (collagens), as well as enzymes (esterases) are important for host defense.[9,10,11,12] So the distortion of these proteins could lead to a breakdown of the immune system of the lung, exposing the body to attack by various foreign bodies that could undermine the health of the lung.

CONCLUSION

Exposure to xylene induces histological and histochemical changes on the lung. These changes are duration dependent. laboratory workers that are exposed to xylene fume are to regulate the rate of exposure.

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