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### RESEARCH PAPER

#### THE HISTOLOGICAL EFFECTS OF FORMALDEHYDE VAPOUR ON THE LUNGS

<sup>1</sup>Odinko CD., <sup>2</sup>Oladele AA., <sup>3</sup>Aneasato AP., <sup>4</sup>Olugbenga MA., <sup>5</sup>Oyadonghan GP.

Department of <sup>1</sup>Medical Laboratory Science, <sup>4</sup>Anatomy, College of Medicine, Ambrose Alli University Ekpoma, Edo State, Nigeria; <sup>2</sup>Morbid Anatomy/Forensic Medicine, Obafemi Awolowo University, Ife-Nigeria;

<sup>3</sup>Histopathology, Enugu State University Teaching Hospital, Enugu, Nigeria; <sup>4</sup>Anatomy, Abia State University, Uturu, Abia State, Nigeria.

\*Corresponding Author: [odinkochibuzo@yahoo.com](mailto:odinkochibuzo@yahoo.com)

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#### ABSTRACT

This study was designed to investigate the effects 40% formaldehyde inhalation on the lungs. Twenty adult male albino rats were used for this study and they were subdivided into five groups (A, B, C, D, and E) with each group containing 5 rats. The animals in group A served as control, while groups B, C, D and E served as the test groups exposed to forty percent (40%) formaldehyde for ten (10), fifteen (15), twenty (20) and twenty five (25) days respectively. The animals in each of the groups were sacrificed to harvest the lungs at the end of each stage of the experiment. The harvested lungs were immediately fixed in 10% formalin for 24 hrs before tissue processing was carried out. Lung tissue sections were stained with Haematoxylin and Eosin (H&E) stains and examined microscopically. The histological findings show that exposure to 40% formaldehyde induces pneumonitis, acute lung injury, pulmonary fibrosis, bronchiolar epithelia degeneration, acute purulent bronchitis, cellular pyknosis and chronic lungs injury. Thus, 40% formaldehyde inhalation can induce lungs injury and possibly lung tumors.

**Keywords:** Formaldehyde, Inhalation, Pulmonary histology, Lung injury.

#### INTRODUCTION

In the world day, the active chemical used for embalming is formaldehyde. It is used in embalment to disinfect, preserve and restore a deceased human body to 'life- like' appearance and this process retains tissue initial architecture (Metcalf et al., 1991).

Despite the use of formaldehyde in embalment, the harmful effects can not be overemphasis as it has an apparent deleterious effect that everyone experiences with a different degree of tolerance (Lab Medicine, 2009). Also, according to International Agency for Research on Cancer (IARC), formaldehyde is placed in Group I amongst known carcinogens like asbestos and benzene (IARC, 2006) and there is "sufficient epidemiological evidence that formaldehyde causes nasopharyngeal cancer in humans," (Collins et al., 1997; Gardner et al., 1993; McLaughlin, 1994; Chang and Adami, 2006 ).

Available literatures reveals also that histopathology examination of inhaled formaldehyde, causes systemic (e.g., anaphylaxis) or localized (e.g., contact dermatitis) allergic reactions (Cronin, 1991; Liden *et al.*, 1993; Lindskov, 1982; Andersen and Maibach, 1983; Trattner *et al.*, 1998; Ebner and Kraft, 1991), respiratory tract irritation, bronchioalveolar constriction, lung oedema (Skog, 1950; WHO IPCS, 1989), squamous-cell carcinomas of the nasal cavities, hypersalivation, acute dyspnea, vomiting, muscular spasms, convulsions and deaths, (Nagomy et al., 1979; Bhalla *et al.*, 1991; Kochar *et al.*, 1986).

It has also been suggested that long-term inhalation of formaldehyde may trigger classic IgE-mediated nasal allergy in atopic individuals while (Wilhelmsson and Holmstrom, 1987) Clinically, formaldehyde has also been implicated in dermatitis and skin sensitization among histo-technologists after chronic exposure to formalin (Dapson and Dapson, 2005).

Meanwhile, the effects of formaldehyde inhalation have been shown to be concentration dependent (Monticello, 1990; Kerns *et al.*, 1983). For instance, high concentrations of formaldehyde (10 - 20 ppm) causes marked hyperplasia and squamous metaplasia of the nasal respiratory epithelium, while the concentration-time-response pattern for inhalation of formaldehyde in rats presented cell proliferation, DNA-protein-crosslink's, cytotoxic effects and Carcinogenicity (CIIT 1999).

Studies has also revealed that endogenous concentration of formaldehyde in human blood does not increase (2.77 microgram/gram) after inhalation for 40 minutes of 1.9 ppm formaldehyde (Heck *et al.*, 1985), while according to Casanova *et al.*, (1988) reported that rats, monkeys, and humans metabolize the same concentrations of formaldehyde in the blood without increase by exposure to high airborne concentrations (up to 15, 6 and 2 ppm respectively) (Casanova *et al.*, 1988). This is because of its rapid metabolism in erythrocytes and no increase in tissue concentration of formaldehyde is detectable even moments after exposure; as formaldehyde is oxidized to formate and exhaled as carbon dioxide (Heck *et al.*, 1985).

However, there is paucity of information on the effects of long time exposure of 40% formaldehyde especially during embalming, being the main constituents of embalming fluid. This study therefore, investigates the chronic histological effects of 40% formaldehyde exposure at different periods on the lungs of adult Wister rats.

## MATERIALS AND METHODS

**Study Area:** The study was conducted at Ambrose Alli University animal house in Ekpoma, Edo State.

**Experimental Animal:** Twenty adult male albino rats weighing 160g-200g were procured from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. They were moved to the site of the experiment at which the control and the test animal were kept in different environment for 2 weeks acclimatization. They were fed with standard pellet diet (vital feeds produced by Grand cereals limited Km17, Zawan round about, Jos, Plateau State, Nigeria) and were provided with clean water. Animals were housed under a controlled room temperature of about 25-28°C and photo-periodicity of 12h day/ 12h night. All animals were treated in accordance with the guide for the care and use of Laboratory Animals prepared (Ochei and Kolhatkar 2000).

**Research Design:** The animals were assigned into five groups (A , B , C , D , and E), containing 4 rats in each group. Group A serves as the control group and groups B-E serves as the experimental groups. All animals were fed with pellitised diet and clean water throughout the experimental period. However the control group (group A; n=4) were not exposed to forty (40%) formaldehyde as they were kept at separate environment while the test animal (groups B-E) were exposed to 40% formaldehyde vapour at a separate period of time and environment (40% formaldehyde at room temperature). The exposures of 40% formaldehyde was conducted by soaking it in cotton wool and placed in enclosed (designed) wire gauze within the animal cage, thus exposing the animals to the vapour. It was exposed for 10, 15, 20 and 25 days in group B, C, D and E respectively. **Sample Collection and Analysis:** The animals were sacrificed at the expiration of the exposure period and the thoracic cage was cut open and the lungs were collected. It was fixed with 10% formalin by immersing in high volume of the fixative and injects the fixative in the lungs with syringe. It was histologically processed within two days after sacrificing.

**Histological Analysis:** The tissues were processed using automatic tissue processor as described in Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-ife, Osun State, Nigeria. It was stained with Haematoxylin & Eosin technique. The slides were examined under a light microscope and photomicrographs were taken.

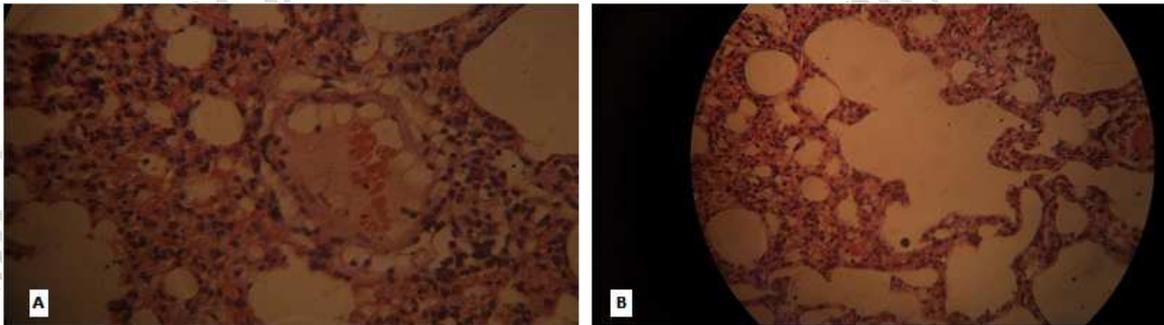
## RESULTS

Group A micrographs (control) showed lung tissue sections with normal pulmonary cytoarchitecture (see plate 1A & B). Micrographs obtained from the group B (10 days) test rats' lung sections showed distortions in pulmonary cytoarchitecture in **plate 2** (A,B,C,D) which includes alveolar wall disruptions, alveolar hemorrhage/edema,

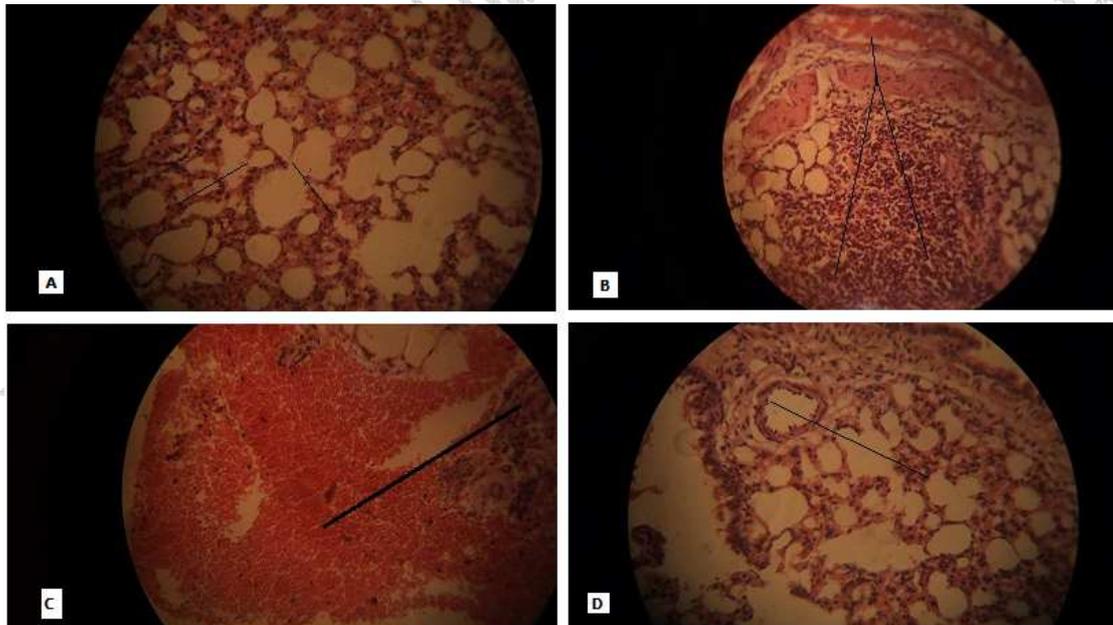
inflammatory cell infiltration, foamy macrophages accumulation, bronchiolar epithelia degeneration, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls and type II pneumocytes hyperplasia.

Micrographs obtained from the group C (15 days) test rats' lung sections showed distortions in pulmonary cytoarchitecture in **plate 3** (A,B,C,D) which includes prominent foamy macrophages accumulation, presence of purulent exudates in the bronchioles, alveolar wall disruptions, alveolar hemorrhage/Edema, inflammatory cell infiltration, hyaline membrane formation, bronchiolar epithelia degeneration, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls and type II pneumocytes hyperplasia.

Micrographs obtained from the group D (20 days) test rats' lung sections showed distortions in pulmonary cytoarchitecture in **plate 4** (A,B,C,D) which includes prominent foamy macrophages accumulation, presence of purulent exudates in the bronchioles, alveolar wall disruptions, alveolar hemorrhage/Edema, inflammatory cell infiltration, cellular pyknosis, hyaline membrane formation, bronchiolar epithelia degeneration, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls and type II pneumocytes hyperplasia.



**Plate 1(A &B):** Lung section (H&E x400) showing normal pulmonary cytoarchitecture.



**Plate 2(A, B, C, D):** Lung section (H&E x400) showing alveolar wall disruptions (plate A), showing alveolar hemorrhage/edema (plate B), inflammatory cell infiltration (plate C), and bronchiolar epithelia degeneration (plate D)

Micrographs obtained from the group D (20 days) test rats' lung sections showed distortions in pulmonary cytoarchitecture in **plate 4** (A,B,C,D) which includes prominent foamy macrophages accumulation, presence of purulent exudates in the bronchioles, alveolar wall disruptions, alveolar hemorrhage/Edema, inflammatory cell infiltration, cellular pyknosis, hyaline membrane formation, bronchiolar epithelia degeneration, pulmonary fibrosis with thickened alveolar walls, enlarged alveolar walls and type II pneumocytes hyperplasia.

**Summary:** Exposure to 40% formalin induced changes in the histology of the lungs and the changes observed were duration dependent.

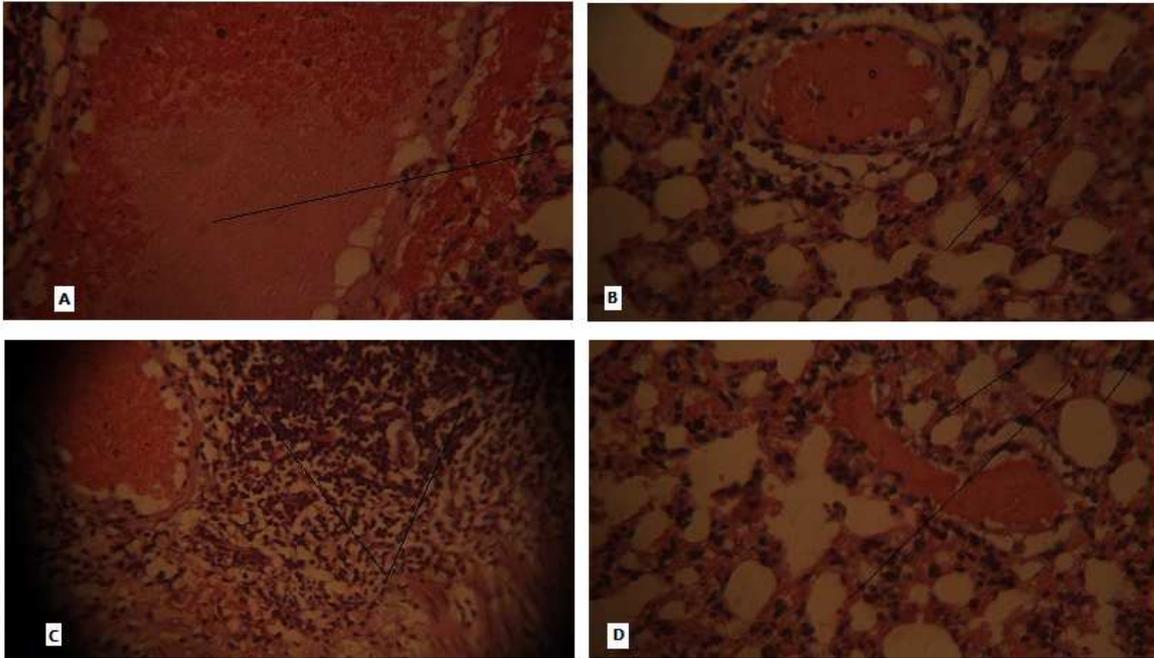


Plate 3(A, B, C, D): Lung Section (H &E x400) showing presence of purulent exudates in the bronchioles (plate A), alveolar wall disruptions (plate B), inflammatory cell infiltration (plate C), and hyaline membrane formation (plate D).

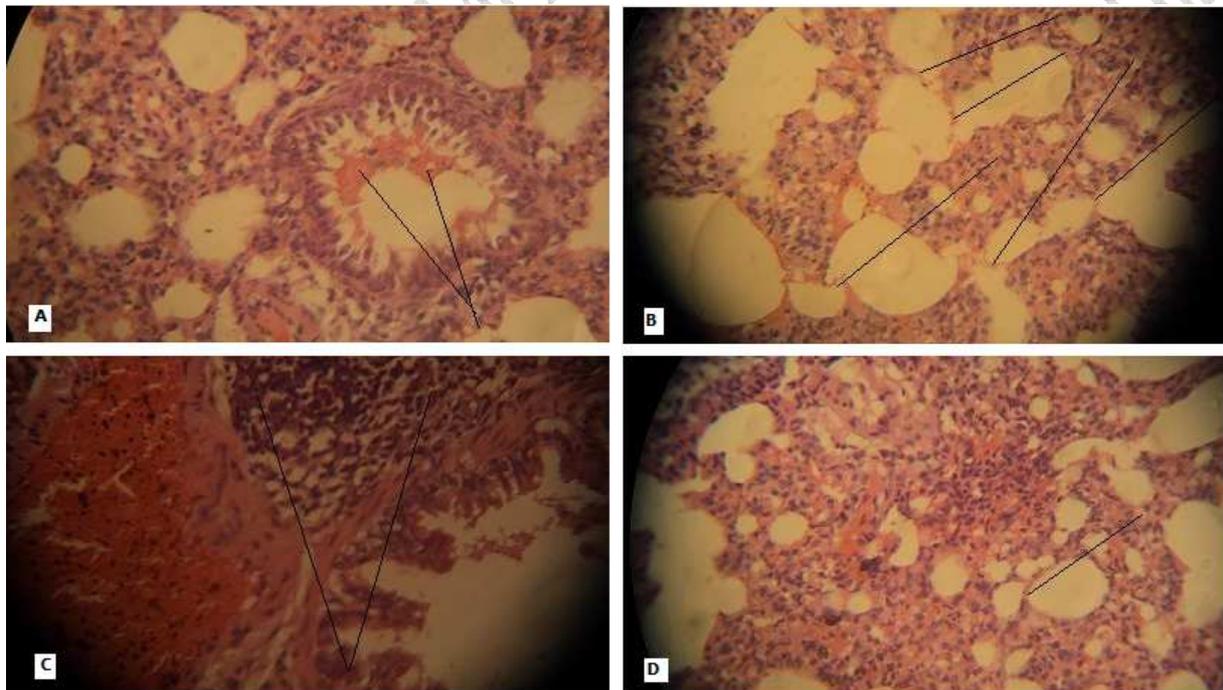


Plate 4 (A, B, C, D): Lung section (H&E x400) showing presence of purulent exudates on the bronchiole (plate A), alveolar wall disruptions (plate B), inflammatory cell infiltration (plate C), and hyaline membrane formation (plate D).

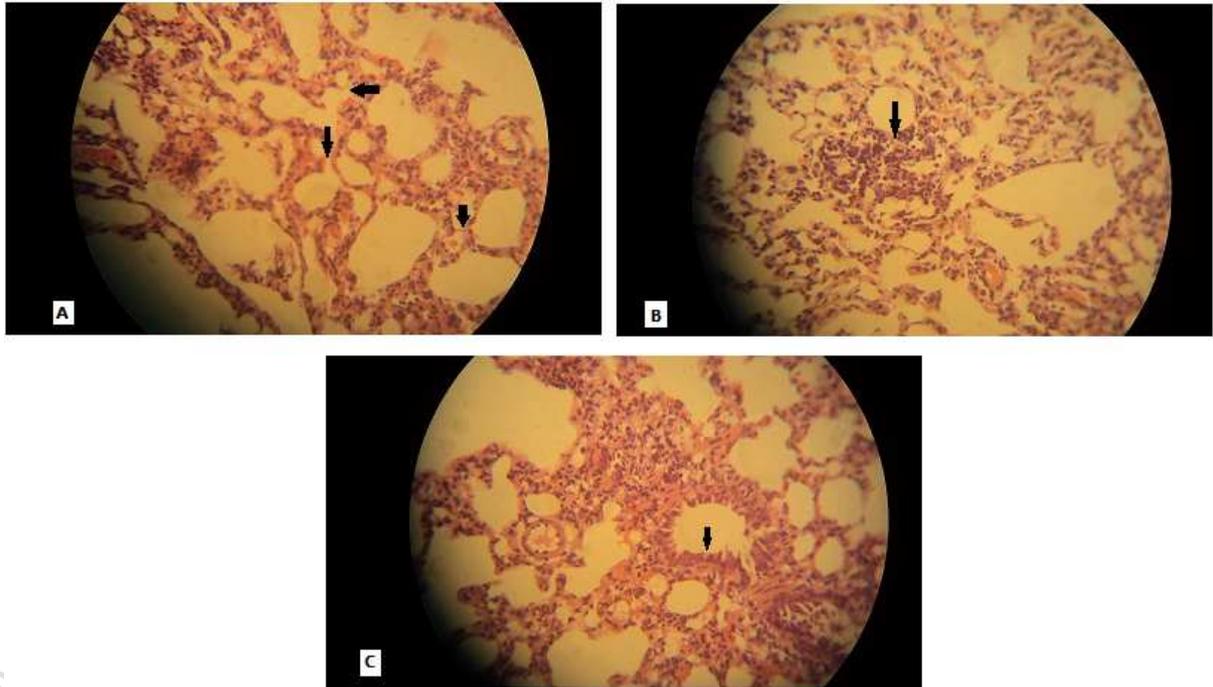


Plate 5 (A, B, C, D): Lung section (H&E x400) showing the presence of alveolar wall disruptions (plate A), inflammatory cell infiltration (plate B), and bronchiolar epithelia degeneration (plate C).

## DISCUSSION

The observed concentration-duration dependent changes in the cytoarchitecture of the lungs suggest that formaldehyde is toxic to the pulmonary cells and the observed histological alterations are in line with the reports by Kamta *et al* (1996), Skog, (1950), and WHO IPCS, (1989), but contradicts that by Kerns *et al*, (1983), that in various chronic studies, no histological changes were found in the lungs following formaldehyde exposure.

Also, the comparative observations on alveolar wall disruptions, haemorrhage, oedema, and cell infiltrations in the test groups, agrees with the reports by Rusch *et al.*, (1983), Wilmer *et al.*, (1987) and (1989), that formaldehyde exposure led to a concentration-dependent epithelial damage in the nasal cavity of rats. Similarly, the result of this study is supported by the reports by Monticello, (1990), Helen *et al.*, (2009), and Salem and Cullumbine, (1960), who reported that lesions developed in the nasal cavity following a high concentration formaldehyde exposure, and that the severity increased with prolonged exposure (duration dependent).

On the other hand, the observed cellular infiltration in the lungs might be as a result of body's immune system reaction to counter the effects of 40% formaldehyde inhalation. Consequently, it may account for the observed progressive disappearance of parenchymal haemorrhage/oedema particularly in group D that is in line with the findings by Binawara *et al.*, (2010), that formaldehyde exposure results in a statistically high significant decrease in the values of FCV, FEV and PEF, which however, reverts back to normal within 24 hrs. Meanwhile the observed cellular infiltrations and epithelial cell degenerations, reaffirms the tumorigenic potentials of formaldehyde, since, according to Woutersen *et al.*, (1989), it can trigger tumour formation based on its inherent capacity to induce epithelial degeration and cell proliferation.

Therefore, our findings suggest that a prolonged high concentration formaldehyde exposure should be avoided as it is potentially deleterious to the lungs, while the adoption of strict safety measures is strongly recommended.

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#### **AUTHOR(S) CONTRIBUTION**

Odinko, C.D. and Oladele, A.A., are the principal investigators. Aneasato AP., Olugbenga MA., and Oyadonghan GP., provided the necessary assistant and support in the research work. All authors were involved the successful presentation of this article.