Section: Anatomy

Original Research Article

EFFECTS OF FORMALDEHYDE ON WISTAR ALBINO RAT LUNG FOLLOWING EXPOSURE THROUGH INHALATION- HISTOLOGICAL STUDY

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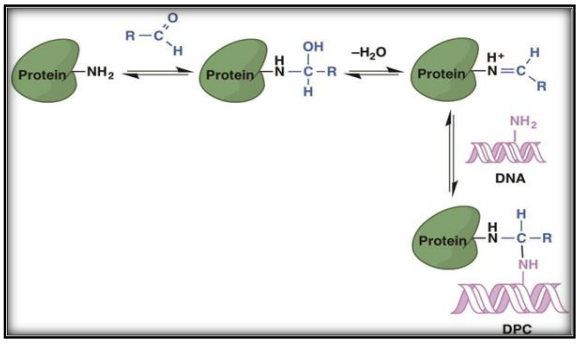
Abstract

Background: Formaldehyde, a flammable, colorless gas, is commonly used in embalming fluid and medical professionals' dissections. This study aims to assess histological changes in albino rats' alveoli architecture after inhalation of formaldehyde vapors. The objective is to observe the histopathological changes in the lungs of Wistar albino rats following exposure to formaldehyde through inhalation. **Materials and Methods:** The study at Kilpauk Medical College, Chennai, involved adult Wistar albino rats exposed to formalin solution. After euthanization, the rats were fixed in 10% formalin for 24 hours. They were sacrificed and the lung tissue was taken and studied by using H & E stain. **Result:** Formaldehyde, a potential immunogen and carcinogen, causes inflammation and damage in various organs. The severity of damage depends on concentration, exposure duration, individual sensitivity, and genetic variation. Our study on lungs found that increased concentration and exposure worsen histological changes, including inflammatory infiltration, alveolar space dilatation, interstitial widening, and peribronchiolar fibrosis. **Conclusion:** It may be concluded from the present study that the concentration of formaldehyde can affect significantly the histopathology of the lungs of albino rats.

INTRODUCTION

Formaldehyde, a common chemical in industrial and household products, poses health risks through inhalation exposure. Its gaseous nature makes it a primary target for respiratory toxicity, leading to airway irritation and severe health issues like bronchial asthma and COPD.[1]

Occupational exposure to formaldehyde among workers and professionals in anatomy dissection halls, pathology laboratories, embalming services, museums, and various chemical industries especially textile and furniture, is highly under-reported. Therefore,the most common route is inhalation and direct skin contact and alsowith industrialization, commercialization, and increased density of population in the cities, people are living in spaces with inadequate ventilation and lighting. This causes stagnation and increased concentration of indoor air pollutants, formaldehyde being one of them.[2-5]Hence, formaldehyde an electrophile, forms reversible adducts or irreversible cross-links with macromolecules such as DNA,RNA, and protein- the desirable property for a conventional tissue fixative.[6-8]Thus making it indispensable in embalming, cadaver, and tissue fixation but also responsible for its genotoxicity and carcinogenicity.[9]



Formaldehyde exposure causes oxidative stress by disturbing the pro-oxidantanti-oxidant balance- the mechanism by which it inflicts injury on several organs- the lung being one of them. It causes the

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accumulation of reactive oxygen species including free radicals such as hydroxyl radical (OH-), superoxide (O2 -), and hydrogen peroxide which react with various cellular components to cause cell damage, tissue damage, and systemic damage by triggering an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity, causing elevated levels of IgG and IgE autoantibodies in human serum. Formaldehyde is highly water soluble and easily diffuses into tissues. It is highly reactive and irritates our eyes and respiratory tract when exposed to its vapors. Anatomists and students in the dissection hall are constantly exposed to the vapors of formaldehyde. Studies have been done on the effects of formaldehyde on Wistar albino rats and mice stating that it has cytotoxic effects on the upper and lower respiratory tract, liver, kidney, skin, also on reproductive system, and nervous system.

This study aims to investigate histological changes in Wistar albino rats following controlled inhalation exposure to formaldehyde. The findings could help understand the respiratory effects of formaldehyde and help develop targeted strategies to mitigate its adverse health effects.

MATERIALSANDMETHODS

This animal experimental study was conducted at the Department of Anatomy, Government Kilpauk Medical College, Chennai after obtaining approval from the Institutional Animal Ethics Committee (Reg. No–375/GO/ReBi/S/01/CPCSEA). The duration of the study was for three months from August 2016 to October 2016. The animals required were provided by the Animal House in our institution. Twenty adult Wistar aibino rats, weighing 180+20 gm were used. Figure :1They were maintained at standard laboratory conditions such as room temperature 25-30°C, light-dark cycle for 12 hours each with access to food and water ad libitum.

Figure 1: Adult Wistar Aibino Rats

Calculation of formaldehyde concentration

The aqueous solution of formalin (37% of formaldehyde in water) that is commonly employed in embalming procedures and in the preservation of various viscera in the Department of Anatomy was used in this study. It contains 37 gm of formaldehyde in 100 mL of water (weight/volume). One ppm equals 1 mg/kg. The conversionof weight by volume to Parts Per Million (ppm) was validated using the given formula

Concentration (ppm)=

$$\frac{24.45 \times \text{Concentration (mg/m3)}}{\text{Molecular Weight}}$$

The molecular weight of formaldehyde is 30.03 grams per mole. In this study, 5 mL of formalin solution was diluted in one liter of water. 37% formalin=370 mg/mL. (37gm/100 ml= 37000mg/100ml=370mg/ml) .5 mL of 37% formalin=1850 mg in 5ml. 5 mL dissolved in 1 L of water=1850 mg/L or 1.85mg/m3 (1 m3=1000L). Using the conversion formula=24.45×1.85/30.03 equals 1.5 ppm. . Hence, 5 ml of formalin solution dissolved in 1 L of water was used for 1.5 ppm exposure groups (E1a, E1b) and 10 mL of the same was used for 3 ppm exposure groups (E2a, E2b). Alternately, Kaden DA et al. had directly given the conversion factors as 1 mg/m3 equals 0.814 ppm. Hence, 1.85 mg/m3 equals 1.505 ppm. Formalin treatment procedure: The experiment group animals were placed in standard cages of 38×20×18 cm size (four animals per cage). The solution was placed in a plastic container with holes in it and kept in the corners of cages. The exposure was carried out in a closed chamber of 55×36×34 cm size. The chamber was modified with openings for light and ventilation at different levels. At the end of the intended exposure period, the rats were euthanized. On cessation of respiration, transcardiac perfusion of 10% formal saline was performed. The lungs were dissected from the surrounding structures, and fixed in freshly prepared 10% formalin.

Methods

Histological study

Tissue processing: Following fixation in 10% formalin for 24 hours, tissue processing steps like dehydration, clearing, paraffin embedding, and serial cut sections in a rotary microtome (6 µm) were done. Then they were stained using Haematoxylin and eosin stains and Masson Trichrome special stain for microscopic evaluation. The control group showed normal architecture of the lung.

Formaldehyde is a ubiquitous chemical compound, a potential immunogen, and a carcinogen. It is found to cause increased inflammation and damage in almost all vital organs. The severity of damage induced depends on the concentration of formaldehyde in the air, duration of exposure, individual sensitivity to the compound, and genetic variation in metabolism. Hence an attempt has been made to study its effects on the lungs. Lung findings of the present study establish the fact that an increase in concentration and duration of exposure causes worsening of the histological changes. Group E1a(1.5ppm,7 weeks) showed inflammatory infiltration alone whereas E2a(3ppm, 7 weeks) showed alveolar space dilatation along

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with inflammatory infiltration. Similarly, E1b(1.5ppm,12 weeks) showed these changes more prominently and additionally showed interstitial widening. E2b (3ppm, 12 weeks) showed further damage with peribronchiolar fibrosis and interstitial fibrosis.

One ppm equals 1 mg/kg. The conversionof weight by volume to Parts Per Million (ppm) was validated using the given formula

$$\text{Concentration (ppm)} = \frac{24.45 \times \text{Concentration (mg/m3)}}{\text{Molecular Weight}}$$

RESULTS

Formaldehydeisa ubiquitouschemical compound, a potential immunogen, and a carcinogen. It is found to cause increased inflammation and damage in almost all vital organs.The severity of damage induced depends onthe concentration of formaldehyde in the air, duration of exposure, individual sensitivity to the compound, and genetic variation in metabolism. Hence an attempt has been made to study its effects on the lungs.Lung findings of the present study establish the fact that an increase in concentration and duration of exposure causes worsening of the histological changes.Group E1a(1.5ppm,7 weeks) showed inflammatory infiltration alone whereas E2a(3ppm,7 weeks) showed alveolar space dilatation along with inflammatory infiltration.Similarly, E1b(1.5ppm,12 weeks) showedthese changes more prominentlyand additionally showed interstitial widening. E2b (3ppm, 12 weeks) showed further damage with peribronchiolar fibrosis and interstitial fibrosis.

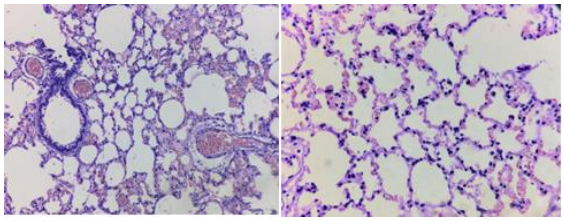

Figure 1: Control-10x normal architecture40x normal alveolar epithelium

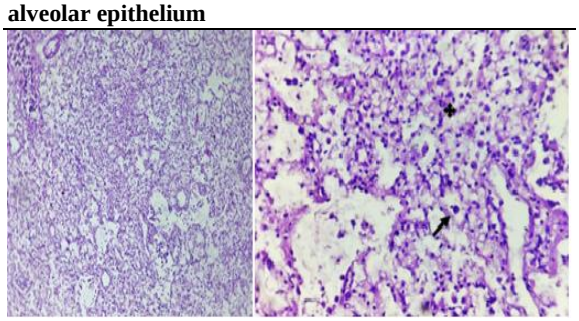

Figure 2: 10x inflammatory infiltration and macrophages. 40x arrow indicating macrophage and mark indicating lymphocytes

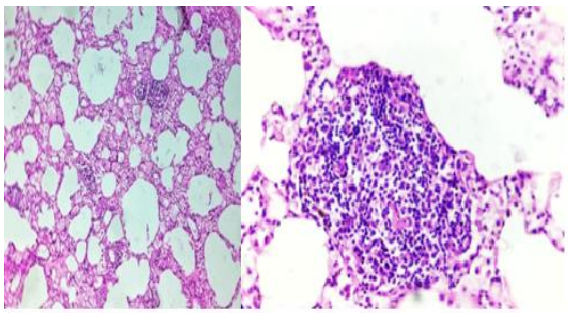

Figure 3: 10X alveolar space dilatation40X inflammatory infiltration

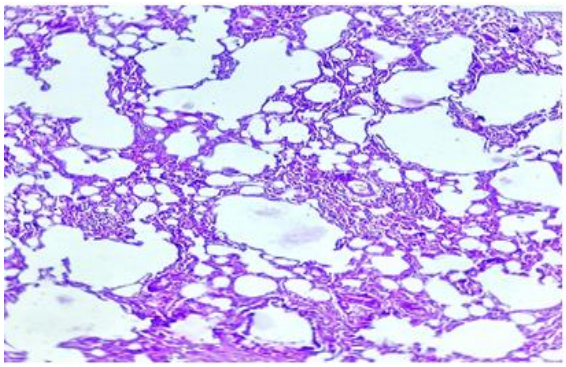

Figure 4: ?

Table 1:

Histological changes	E1a 1.5ppm 7weeks	E2a 3ppm 7weeks	E1b 1.5ppm 12weeks	E2b 3ppm 12weeks
Inflammatory infiltrate	+	++	++	++
Alveolar space dilatation(emphysema)		+	++	++
Interstitial widening			+	+
Bronchiolar Epithelial desquamation				+
Peribronchiolar fibrosis				+
Interstitial fibrosis				+

DISCUSSION

From the observations of the present study, it is very clear that formaldehyde causes damage to the bronchiolar epithelium and alveolar epithelium with architectural distortion which is consistent with the findings of previous studies Sezgin aydemir et al, Asmaa M.T. Mohamed et al, Ahmed Hamdi et al.,

In another study P. Saxena, Archana Sharma examined the histological changes in the alveoli of albino rats exposed to formaldehyde vapors.[10-12] The rats were exposed to various concentrations of formaldehyde for 28 days, and their lung tissue was studied using an H&E stain.[13,14] The results showed concentration-dependent changes, suggesting that the concentration of formaldehyde can significantly

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affect the histopathology of the lungs of albino rats. A similar study by Yu-Hua Yanganet al., analyzed protein changes in Wistar rats exposed to gaseous formaldehyde (FA) for 4 hours daily for 15 days.[15,16] Four proteins were significantly altered, with three up-regulated and one down-regulated. These proteins are related to cell proliferation and anti-oxidation defense reactions. Proteomics is a valuable tool for environmental health research and disease diagnosis and monitoring.[17] One more study S. Çıkmaz, T. Kutoğluinvestigated the effects of formaldehyde (FA) on the liver in rats, using 18 Wistar albino rats exposed to different levels of FA gas.[18] The liver tissue samples showed enlarged sinusoids, infiltration, loss of cytoplasm, and hyperchromatic nucleus in FA-exposed rats. The cells of FA-exposed livers showed an electron-lucent ground-cytoplasm and hypertrophy of the smooth-surfaced endoplasmic reticulum. The study found that FA exposure caused diverse histopathological changes, with destruction in liver tissue directly related to exposure duration.[19]

Limitation

The formaldehyde exposure and concentration in parts per million were calculated using the conversion formula. The exact concentration in parts per million of formaldehyde in air in real-time following formalin solution exposure could not be determined because of the unavailability of a gas chamber with a detector tube.

CONCLUSION

Formalin asthma and sick-building syndrome are terms synonymous with formaldehyde exposure. The study showed that exposure to even minimal doses of formaldehyde such as 3ppm can have adverse effects on the lungs including emphysematous changes, interstitial widening, bronchiolar epithelial desquamation, and peribronchiolar fibrosis. The present study attempted to raise concerns regarding the lighting and ventilation strategies in the anatomy dissection hall and pathology laboratory and another factory environment that can control the concentration of formaldehyde in air and thus reduce its ill effects. Melatonin, omega 3 fatty acid, vitamin E, and Nigella Sativa through their antioxidant properties have been shown to have some protective effects against the damage produced by formaldehyde-prospects of this study.

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