ABSTRACT

Introduction: Formaldehyde is a flammable, colourless, reactive, readily polymerized gas at normal room temperature and pressure. Formaldehyde vapour is readily absorbed from the lungs. Formaldehyde is combined with methanol and buffers to make embalming fluid. In the dissection hall, during embalming or cadaveric dissection and histo-pathological preservation, medical professionals are exposed to formaldehyde vapours.

Respiratory system is the major target of formaldehyde. So, the present study aims to assess the histological changes on the architecture of alveoli of albino rats after inhalation of formaldehyde vapours.

Material & Methods: 24 albino rats were exposed to formaldehyde vapours for 28 days. They were exposed with various concentrations and thus 4 groups, having 6 rats in each, were made. They were sacrificed and the lung tissue was taken and studied by using H & E stain.

Results: The study shows a number of important histological changes which are concentration dependent.

Conclusion: It may be concluded from the present study that concentration of formaldehyde can affect significantly on the histopathology of the lungs of albino rats.

Keywords: Formaldehyde exposure, lung alveoli architecture, histo-pathological changes.

INTRODUCTION

Formaldehyde is the simplest aldehyde which is a flammable, colourless, reactive, readily polymerized gas at normal room temperature and pressure, with a relative molecular mass of 30.03 and a pungent odour [1]. Formaldehyde concentration is generally explained as parts per million (ppm; 1 ppm¼ 1.25 mg/m³). It is a strong-smelling gas which is often found in aqueous solutions called formalin solution, with a maximum concentration of 37% by weight (40% by volume). Formalin solutions often contain some amount of methanol (10 -15% v/v) as a stabilizer to prevent polymerization of formaldehyde. Fixative solutions labelled as 10% buffered formalin are actually 4% solutions of formaldehyde (a 10% solution made from a 37- 40% solution of formaldehyde) [2]. Considerable human exposure to formaldehyde gas occurs at concentrations up to 1 ppm [3].

In the dissection lab, during embalming, cadaveric dissection and histo-pathological preservation, medical professionals are exposed to formaldehyde vapours.
Formaldehyde is readily absorbed from the respiratory tract, from the gastrointestinal tract after ingestion and gives serious systemic effects. It gives local effects by ocular absorption of vapours like irritation, burning sensations and lacrimation. It is poorly absorbed following dermal exposure [4]. The commonest route of exposure of formaldehyde is by inhalation and even fairly low concentrations of formaldehyde can produce rapid onset of nose and throat irritation, causing cough, chest pain, shortness of breath, and wheezing. Higher exposures can cause significant inflammation of the lower respiratory tract which can lead to haemorrhagic to fibrotic changes [5]. Respiratory system is the major target of formaldehyde. So, the present study was undertaken to assess the histological changes on the architecture of alveoli of albino rats after inhalation of formaldehyde vapours at different concentration.

MATERIAL AND METHODS

The present study was conducted after obtaining approval from the Institutional Ethical Committee. 24 albino rats of both sexes, weighing approximately 150-200 grams were used in the experiment. The rats were housed in a well-lighted and ventilated room. Under controlled condition, they were acclimatized for 3 weeks before the experiment. Fifty grams of food in the form of pellets per day and water ad libitum was given to the rats.

The study comprised of 4 groups of albino rats. Group I was not exposed with formaldehyde and taken as Control, Group II was exposed at 4%, Group III at 8% and Group IV was exposed at 40% formaldehyde solution till 28 days.

For exposure, 40 ml of formaldehyde solution was kept in a petridish in the cage for 4 hours per day, 6 days a week. After the experiment was over for a particular group, the rats were taken, weighed, anaesthetized, and then dissected. The thoracic cage was opened and the lungs were taken out and preserved. After fixation, dehydration, clearing, wax impregnation and embedding was done. Then ribbon sections were made and staining procedure was done with Hematoxylin and Eosin stain. Further the slides were observed under microscope for histological study.

OBSERVATIONS AND RESULTS

In the present study remarkable increase in histopathological changes in lungs of albino rats were observed as compared to control group. The Hematoxylin and Eosin stained sections of lungs of control rats showed no obvious histopathological changes (Fig. 1).

Among the experimental rats, the findings are detailed with their presence as the number of rats among 6 rats of particular group (Table 1, Fig. 2-11).
Table 1: Number of rats in each group in which particular histological finding is present

| S.No. | FINDINGS                              | I (C) 6rats | II (4%) 6 rats | III (8%) 6 rats | IV (40%) 6 rats |
|-------|--------------------------------------|-------------|----------------|-----------------|----------------|
| 1     | Alveolar wall thickness               | 0           | 6              | 6               | 6              |
| 2     | Alveolar space narrowing              | 0           | 6              | 6               | 6              |
| 3     | Alveolar wall disruption              | 0           | 6              | 6               | 6              |
| 4     | Emphysema                             | 0           | 3              | 3               | 4              |
| 5     | Distorted interstitial architecture   | 0           | 6              | 6               | 6              |
| 6     | Alveolar edema                        | 0           | 6              | 6               | 6              |
| 7     | Alveolar hemorrhage                   | 0           | 6              | 6               | 6              |
| 8     | Dilatation and congestion of blood vessels | 0           | 6              | 6               | 6              |
| 9     | Pulmonary vasculitis                  | 0           | 3              | 4               | 4              |
| 10    | Thickening of blood vessel wall       | 0           | 4              | 4               | 5              |
| 11    | Inflammatory infiltrates              | 0           | 6              | 6               | 6              |
| 12    | Lymphoid tissue Hyperplasia           | 0           | 5              | 6               | 6              |
| 13    | Fatty infiltrates in pulmonary interstitium | 0           | 3              | 4               | 4              |
| 14    | Pulmonary fibrosis                    | 0           | 3              | 3               | 4              |

Fig. 3: A photomicrograph of lung of albino rat (4 weeks of exposure of 8% formaldehyde). H&E X 400. Showing Alv. wall thickness, Alv. space narrowing, Alv. wall disruption (Arrow heads), Alv. hemorrhage, distorted interstitial architecture, inflammatory infiltrates

Fig. 4: A photomicrograph of lung of albino rat (4 weeks of exposure of 8% formaldehyde). H&E X 100. Showing Alv wall thickness, Alv space narrowing, Alv wall disruption (Arrow heads), Alv hemorrhage, distorted interstitial architecture
Fig. 5: A photomicrograph of lung of albino rat (4 weeks of exposure of 40% formaldehyde). H&E X 100. Showing alveolar wall thickness, Alv. space narrowing, Alv. wall disruption (Arrow heads), emphysematous bullae (EB), distorted interstitial architecture.

Fig. 6: A photomicrograph of lung of albino rat (4 weeks exposure of 4% formaldehyde). H&E X 400. Showing Alv wall thickness, Alv wall disruption (Arrow heads), distorted interstitial architecture, inflammatory infiltrates, Alv. edema (AE) and Alv. haemorrhage (AH).

Fig. 7: A photomicrograph of lung of albino rat (4 weeks exposure of 8% formaldehyde). H&E X 100. Showing Alv wall thickness, Alv space narrowing, distorted interstitial architecture, inflammatory infiltrate, Alv haemorrhage (AH), pulmonary vasculitis (PV).

Fig. 8: A photomicrograph of lung of albino rat (4 weeks exposure of 40% formaldehyde). H&E X 100. Showing distorted architecture, alveolar haemorrhage (AH), dilatation and congestion of blood vessels (BV), thickening of blood vessel wall (arrow heads).

Fig. 9: A photomicrograph of lung of albino rat (4 weeks exposure of 4% formaldehyde). H&E X 100. Showing distorted architecture, dilatation of blood vessel, inflammatory infiltrates, lymphoid tissue aggregation (arrow head), fat cells infiltration (FC).

Fig. 10: A photomicrograph of lung of albino rat (4 weeks exposure of 8% formaldehyde). H&E X 100. Showing dilatation and congestion of blood vessel (BV), inflammatory infiltrates, lymphoid tissue aggregation (LT), foci of fibrosis (F).
Histological effects of formaldehyde...

Fig. 11: A photomicrograph of lung of albino rat (4 weeks exposure of 40% formaldehyde). H&E X 100. Showing distorted interstitial architecture, Alv. edema (AE) and Alv. haemorrhage (AH), inflammatory infiltrates, foci of fibrosis (F).

STATISTICAL ANALYSIS

For statistical significance, on applying the Fischer exact test we have seen that the exposure of formaldehyde for 28 days was significantly associated (p value < 0.05) with the alveolar wall thickness, alveolar space narrowing, alveolar wall disruption, distorted interstitial architecture, alveolar edema, alveolar haemorrhage, dilatation and congestion of blood vessels, inflammatory infiltrates and lymphoid tissue hyperplasia at all 4%, 8% as well as 40% concentration of formaldehyde. Further, we noted that emphysema, pulmonary vasculitis, thickening of blood vessel wall, fatty infiltration in pulmonary interstitium and pulmonary fibrosis was not significantly associated (p value > 0.05) at any concentration of formaldehyde on 28 days of exposure of formaldehyde (Fig. 12).

Fig. 12: Histogram showing comparison between experimental groups with no. of rats affected by different concentration of formaldehyde solution.
DISCUSSION

It is evident in the present study that the exposure of formaldehyde solution resulted in distinctive alteration of architecture of the lungs of albino rats. Table 1 is showing the number of rats in which particular finding is present in all groups.

Acute lung injury (ALI) (also called non cardiogenic pulmonary edema) is characterised by the abrupt onset of significant hypoxemia and bilateral pulmonary infiltrates in the absence of cardiac failure. Acute respiratory distress syndrome (ARDS) is a manifestation of severe ALI. Both ARDS and ALI are associated with inflammation-associated increases in pulmonary vascular permeability, edema and epithelial cell death. The histologic manifestation of these diseases is diffuse alveolar damage (DAD). ALI is a well-recognized complication of diverse conditions, including both direct injuries to the lungs and systemic disorders. The pathogenesis of ALI/ARDS is initiated by injury of pneumocytes and pulmonary endothelium, setting in motion a viscous cycle of increasing inflammation and pulmonary damage [6].

In the present study, Alveolar wall thickness that means the increased cellularity of alveolar wall is observed. The interstitial tissue was thickened due to accumulation of inflammatory cells with edema [7]. Blood vessels in the airway proliferate the influence of growth factors such as vascular endothelial growth factors (VEGF) and may contribute to increase airway wall thickness [8]. This may be the reason of alveolar space narrowing.

Alveolar wall disruption or ulceration is observed as a result of the mechanism of excavation and desquamation of the surface epithelium and the supporting tissues of the alveolar wall [9]. Due to injury of wall ultimately the interalveolar septa ruptures and bulla formation occurs and leads to Emphysema. Thus the distorted architecture of pulmonary interstitium is present in all concentrations.

Alveolar edema can be due to increase in capillary permeability as a result of microvascular injury due to formaldehyde inhalation and is present in all the rats of experimental groups. The mechanism of polymorphonuclear leukocytes inflammatory cells invasion induced by formaldehyde inhalation can be explained as inhaled formaldehyde rapidly increases vascular permeability in rat airway and produces microvascular leakage in the airway through stimulation of tachykinin NK1 receptors by tachykinins released from sensory nerves [10]. This ongoing inflammation leads to alveolar haemorrhage and dilatation and congestion of blood vessels. In this sequence, pulmonary vasculitis was prominently seen along significant thickening of blood vessels.

Whenever there is exposure to some noxious substance like formaldehyde, body's immune system reacts to counter the effects, as a result there is cellular infiltration in the lungs [11]. As a result of cellular injury, inflammatory infiltrates were present significantly in almost all of the tissue sections along with lymphoid tissue proliferation. In some of the sections fatty infiltration was also there.

When the exposure occurs consistently, there is ongoing process of injury, tissue destruction and attempts for healing by connective tissue forming foci of fibrosis. This fibrosis is observed in a number of sections, especially exposed with higher concentration.

These findings are also present in the studies done previously and they are compared with the present one (Table 2).

CONCLUSION

In the present study, we found that the exposure of formaldehyde affects significantly on the histopathology of lungs. The severity of findings increases with the increase in concentration of exposure.

As the formaldehyde is one of the cause of asthma and it can do airway inflammation with harmful changes as in young smokers, it can be clearly said that we are equally prone to diseases like asthma and respiratory distress syndrome. So this knowledge is very essential for us as anatomists, pathologists, embalmers, technicians, medical and veterinary student to be cautious of the harmful effects associated with formaldehyde inhalation.
| FINDINGS                          | Sheela, Sreedevi [12] (1991) | Turkoglu et al [13] (2008) | Njoya et al [9] (2009) | Bansal et al [7] (2011) | Mohamed et al [14] (2012) | Odinko et al [15] (2012) | Mehdi et al [16] (2014) | Chinedum et al [17] (2014) | Uche et al [11] (2015) | Afrin et al [18] (2016) | Verma et al [19] (2016) | Cheng et al [20] (2016) | Present study (2018) |
|----------------------------------|-------------------------------|-----------------------------|-----------------------|-------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------------------|
| Alveolar wall thickness          | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |
| Alveolar space narrowing         |                               |                             |                       |                         |                           |                         |                         |                         |                         |                         |                         | √                     |
| Alveolar wall disruption/ulceration |                           |                             |                       |                         |                           |                         |                         |                         |                         |                         |                         | √                     |
| Emphysematous bullae             | √                             | √                           |                       |                         |                           |                         |                         |                         |                         | √                       | √                       | √                     |
| Distorted interstitial architecture |                               |                             |                       |                         |                           |                         |                         |                         |                         |                         |                         | √                     |
| Alveolar edema                   | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |
| Alveolar hemorrhage              | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |
| Dilatation and Congestion of blood vessels | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |
| Pulmonary vasculitis             |                               |                             |                       |                         |                           |                         |                         |                         | √                       |                         |                         | √                     |
| Thickening of blood vessel wall  | √                             |                             |                       |                         |                           |                         |                         |                         |                         |                         |                         | √                     |
| Inflammatory infiltrates         | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |
| Lymphoid tissue Hyperplasia      | √                             |                             |                       |                         |                           |                         |                         |                         | √                       |                         |                         | √                     |
| Fatty infiltrates in pulmonary interstitium | √                             |                             |                       |                         |                           |                         |                         |                         |                         |                         |                         | √                     |
| Pulmonary fibrosis               | √                             | √                           | √                     | √                       | √                         | √                       | √                       | √                       | √                       | √                       | √                       | √                     |

Table 2: Comparison of the findings of present study with the studies done previously.
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