

Effect of exposure to formalin on the histochemical structure of respiratory organs in Mice**Samah M. Alaa-Eldein^{1*}, Enas A. Hafez², Soha soliman¹, and Mohammed Abdelsabour-Khalaf³**

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Abstract

This study was evaluating histopathological changes in lung tissue induced by formaldehyde vapors in mice. A group of 20 adult male mice was divided into two groups for this purpose. The mice in group (I) are the controls, whereas the mice in groups (II) were exposed to formaldehyde vapors inhalation in a toxic dose (0.5 PPM) every day for two months in the students dissecting room of South Valley University's Faculty of Veterinary Medicine. Lung samples were prepared for light microscopic examination after exposure. Gross structural abnormalities in the lungs were identified, including congestion in most lobes and localized pneumonic organization. Thickening of the alveolar septum, epithelial hyperplasia in the bronchioles, proliferative capillaries, pulmonary vasculitis, and hyperplastic lymphocytic aggregations in the parabronchiolar area, pulmonary fibrosis, and precancerous modifications were detected under light microscopy (metaplasia of goblet cells and dysplasia of the bronchiolar epithelium). It was found that inhaling formaldehyde has an irritating toxic effect in addition to its harmful carcinogenic effect on the lungs of mice, which is related to exposure durations.

Keywords:

Fibrosis, Formaldehyde, Lymphoid hyperplasia, Mice lung.

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Introduction

Formaldehyde is an organic carbon compound that is commonly found in industrial areas and hospitals in addition to many fabrics, papers, wood manufacturing, and resins) as well as in homes (padding materials, chipboard, and cooking vapours), and it has been identified as one of the main causes of sick building syndrome (Kim et al., 2002, Kita et al., 2003, Nakazawa et al., 2005). Formaldehyde is a volatile, colorless, unpleasant-smelling gas with a pungent odor that irritates the eyes and nasal mucous membranes (Nelson et al., 1986). It has been found to be toxic at a certain concentration, and its harmful effects have increased at room temperature due to its volatility, particularly in areas with poor ventilation (Songur et al., 2003). After dissolving in water, it is known as formalin, which is commonly used in medical labs as a disinfectant and sanitizer. It also enters food preservation and food products, such as medications, antiseptics, and cosmetics. Formaldehyde is not used often, but chemicals that release formaldehyde are used. These chemicals can be found in cosmetics, shampoos, soaps, sunscreens, lotions, and cleaning products. It can also be released from smoking or cooking. Formaldehyde has also been detected in the environment. Humans and biological organisms produce small amounts of formaldehyde as part of the body's natural metabolic processes (Tarver, 2012). The most common way humans are exposed to formaldehyde is by inhalation. On the other hand, its liquid form may be absorbed through the skin. Formaldehyde can also enter the body in small amounts through the consumption of foods and drinks containing it (Bosetti et al., 2008). Formaldehyde synthesis occurs naturally in the body and it is broken into formate (formic acid) by enzymes in the body, which is then broken down into

carbon dioxide. Most inhaled formaldehyde is broken down by the cells lining the mouth, nose, throat, and airways, so only about a third of it enters the circulation through absorption (Catalani et al., 2019). After inhaling formaldehyde at concentrations more than 0.1 parts per million (PPM), people may suffer from many health problems, including watery eyes, irritating sensations in the eyes, nose, and throat, cough, breathing difficulties, nausea, and skin burning sensations. Many people are highly sensitive to formaldehyde, whereas others are not affected by the same degree of exposure (Mohamed, 2012). Due to known risks of nasopharyngeal cancer and leukemia, the International Agency for Research on Cancer (IARC) has concluded that formaldehyde is "highly carcinogenic to humans (Checkoway et al., 2015). According to the IARC's expert working group, formaldehyde causes nasopharyngeal cancer in humans and has a cumulative effect in industrial employees. The absolute risk of lung cancer increased significantly (Humans, 2006). As the previous studies only shed lights on the harmful effects of formaldehyde on the upper respiratory tract particularly the nose and much less concern is focused on the pulmonary toxicity, the present study focus on the cellular toxicity resulting from formaldehyde inhalation on the lungs of albino mice.

Materials and methods

Experimental design

In the current study, 20 adult male albino mice weighing 30 -35 gm were used, received from the Animal House, Faculty of Veterinary Medicine, South Valley University. They were kept in stainless steel mesh cages under normal temperature (22-24°C) and humidity standards. Animals had full access to water

and were fed a balanced meal in accordance with animal care guidelines provided by the European Community Guidelines on the Care and Use of Laboratory Animals (Community, 1986). Two groups of mice were formed. The mice in group (I) are the controls, which were kept in another room without any exposure to formaldehyde. Whereas the mice in groups (II) were exposed to formaldehyde gas inhalation in a toxic dose (0.5 PPM) every day for two months in the students dissecting room of South Valley University's Faculty of Veterinary Medicine. The source of Formaldehyde gas in the dissecting room is the large containers in which animal carcasses were preserved in 40% formaldehyde solution (which is usually prepared by dilution of the commercial formalin with ordinary tap water in dissecting rooms to preserve cadavers). The exposure time is 4h / day for 5 days / week until week 8. (2months). This experimental design was done as a simulation for the situation in the university, especially among the students of the first and second year of veterinary medicine, who are studying anatomy and spending time in the dissecting rooms and pathology laboratories, in which carcasses of animals are kept in formaldehyde solution. Being there for a long time and being exposed to formaldehyde vapors is extremely toxic.

Methods

Animals were anaesthetized with ether inhalation at the end of the study and after the last formaldehyde exposure. Following thoracotomy, the lungs were gently dissected out and examined for any changes in shape, size, color, or consistency with the naked eye. The lungs were then preserved in 10 % formaldehyde before being processed for light microscopy (Bancroft and Stevens, 1990). Sirius Red

stain technique is also used to identify the interstitial fibers of the control lung and Detection of pulmonary fibrosis in the treated group (Kiernan, 2011).

Results

For accurate understanding of this study, the results were summarized according to clinical observations, anatomical and histological results into the following:

Animals were healthy by the beginning of the study. The clinical signs of the mice in the control group were normal. On the other hand, Mice exposed to formaldehyde showed mild dyspnea, listlessness, and a hunched posture, which became more prominent with time and were accompanied by additional clinical signs such as mouth breathing, loss of skin elasticity, ataxia, sensory irritation (reduced breathing frequency), ocular irritation including (mild lacrimation and conjunctival hyperemia). After a week of experimentation, those clinical signs began to appear. Dullness, staggering gait, sitting with closed eyes, and decreased responsiveness to disturbance in addition to decreased feed and drink intake.

Anatomical results

The morphological appearance of the control group's lungs (I) revealed a normal rosy pink color of the studied lung. There were no noticeable changes in size or consistency. In both lobes, the lungs showed smooth normal borders and typical ventral and dorsal surfaces, with no air cystic changes or pathological masses. The different parts of the lung tissue were well differentiated in cut (transverse) sections. The conducting part (trachea and bronchi) was also examined and found to be normal in appearance and color (Fig. 1A).

On the other hand, there were many morphological changes in lungs of the exposed group that showed irregular thin

borders of most lobes of the lung and severe congestion and hyperemia in all lobes, which appeared in the form of dark red color in lung tissues. Furthermore, smooth swollen surfaces were present, along with an increase in the volume of the examined lung lobes, indicating edema which is confirmed by cut sections and presence of a serous fluid oozing from lung tissue. (Fig. 1 B).

The lung also showed irregular convoluted surfaces. The presence of

significant lung vasculature is indicated by increased congestion in all lobes and increased pulmonary vascular marking. The lungs' borders and sizes were irregular, and there were small red hemorrhagic spots in the upper lobes of both lungs. Focal pneumonic organization of the pulmonary tissue was clear, as demonstrated by developed fibrotic areas of the pulmonary tissues. Three compressed pitting depressions can be seen on the ventral surface of the right upper lobe, and the left lobe is completely enlarged (Fig. 1 B).



Fig. 1 :(A) the lungs of the control group, (B) lungs of the exposed group.

Histological results

Light microscopic examination

Control group

Lung tissues were used to study the normal histological features of mice lungs. Sections of the lungs were stained with the conventional H/E and examined via light microscope. The light microscopic examinations showed no pathological alterations in the histological structures of the lung. It revealed typical lung tissue architecture, including the alveolar sacs, alveolar ducts, and alveoli, as well as clear identifiable bronchiolar passages and

alveolar cavities. The respiratory bronchioles continued as alveolar ducts. The alveolar ducts opened into numerous alveolar sacs (Fig.2 A).

The bronchiolar mucosa thrown into numerous longitudinal folds and was easily recognized into lamina epithelialis and lamina propria. The bronchiolar epithelium rested on a thin lamina propria that consisted of fine network of elastic fibers, reticular fibers and collagen fibers. The bronchiolar mucosa was surrounded by thin band of smooth muscle fibers and supported by peribronchiolar connective tissue

contained diffuse lymphatic tissue (Fig. 2 B).

Normally, pulmonary vessels were scattered throughout the pulmonary parenchyma (Fig.2A). The alveolar septa were normal in thickness, with no abnormalities in the blood capillaries (Fig. 1B).

The mucosa of the terminal bronchiole was formed of lamina epithelialis and lamina propria and lacked longitudinal folds. Simple cuboidal cells were interspersed between apical dome-shaped cells that protruded into the airway lumen in the lamina epithelialis (Fig. 2 C).

The respiratory epithelium of the alveoli, which includes simple squamous epithelial cells interspaced by simple cuboidal cells, was also seen at a higher magnification. Type I pneumocytes and Type II pneumocytes were the former cells. Type II pneumocytes are found in the angular junctions of alveolar walls. The cytoplasm of alveolar macrophages was foamy pale stained, and the cells were spherical. Alveolar ducts have thin bands of

smooth muscle fibers and a network of collagen and elastic fibers on their walls (Fig. 2 D).

Formaldehyde exposed group Inflammatory and Fibrotic changes of the lung (pneumonia and pulmonary fibrosis) in the conducting portion.

Bronchiolitis was identified by the presence of diffuse inflammatory cells in the peri-bronchiolar tissue (Fig. 3 A, B). Bronchiolar epithelial sloughing or desquamation was identified associated with *Bronchiolitis* (Fig. 3 A). Intra-bronchiolar hemorrhage associated with sever *bronchiolitis* (Fig. 3 C, D). Sever *bronchiolitis* associated with massive infiltration of inflammatory cells (Fig. 3 E, F).

Pulmonary fibrosis was detected at various degrees. Mild fibrotic changes were recognized in the peri-bronchiolar tissue (Fig. 3 A). Extensive fibrosis was identified at the inflammatory site associating with *Bronchiolitis and vasculitis* (Fig. 3 B-D, F). Fibrotic changes were also observed associated with extensive peri-bronchiolar edema (Fig. 3 E).

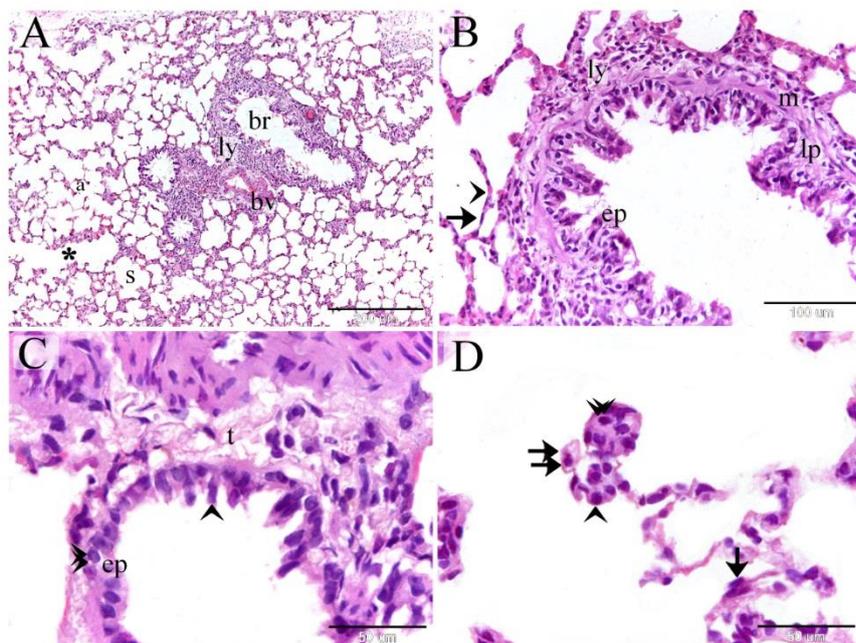


Fig. 2. Control sample of the lung stained by H&E

Fig. 2. Control sample of the lung stained by H&E: Paraffin sections of control samples of the lung tissues showed normal pulmonary tissue architecture. A: clear obvious bronchiolar passages (br) and alveolar cavities including the alveolar sacs (s), the alveolar ducts (asterisk) and the alveoli (a). Pulmonary vessels (bv) were normally scattered within the pulmonary parenchyma. B: The bronchiolar mucosa thrown into numerous longitudinal folds and was easily recognized into lamina epithelialis (ep) and lamina propria (lp) which was layer of connective tissue that consisted of fine network of elastic fibers, reticular fibers and collagen fibers. The bronchiolar mucosa was surrounded by thin band of smooth muscle fibers (m) and supported by peribronchiolar connective tissue contained lymphoid tissue (ly). The alveoli comprised a part of the respiratory portion. Note the alveolar septa (arrow) had normal thickness with no deformity in blood capillaries (arrowhead) of the alveolar septa. C: terminal bronchiole consisted of simple cuboidal epithelium (ep) interspersed between apical dome-shaped cells that protruded into the airway lumen (arrowhead). Note migrating lymphocytes (double arrowhead), peribronchiolar connective tissue (t). D: alveoli were lined by the respiratory epithelium which include simple squamous epithelial cells (arrow) interspaced by simple cuboidal cells (arrowhead). The former cells were the Type I pneumocytes and Type II pneumocytes. Pneumocyte Type II located at the angular junctions of the alveolar walls. Alveolar macrophages (double arrowhead) appeared as rounded cell that had a foamy pale stained cytoplasm. The walls of alveolar ducts also contained thin bands of smooth muscle fibers and network of collagen and elastic fibers (**double arrowhead**).

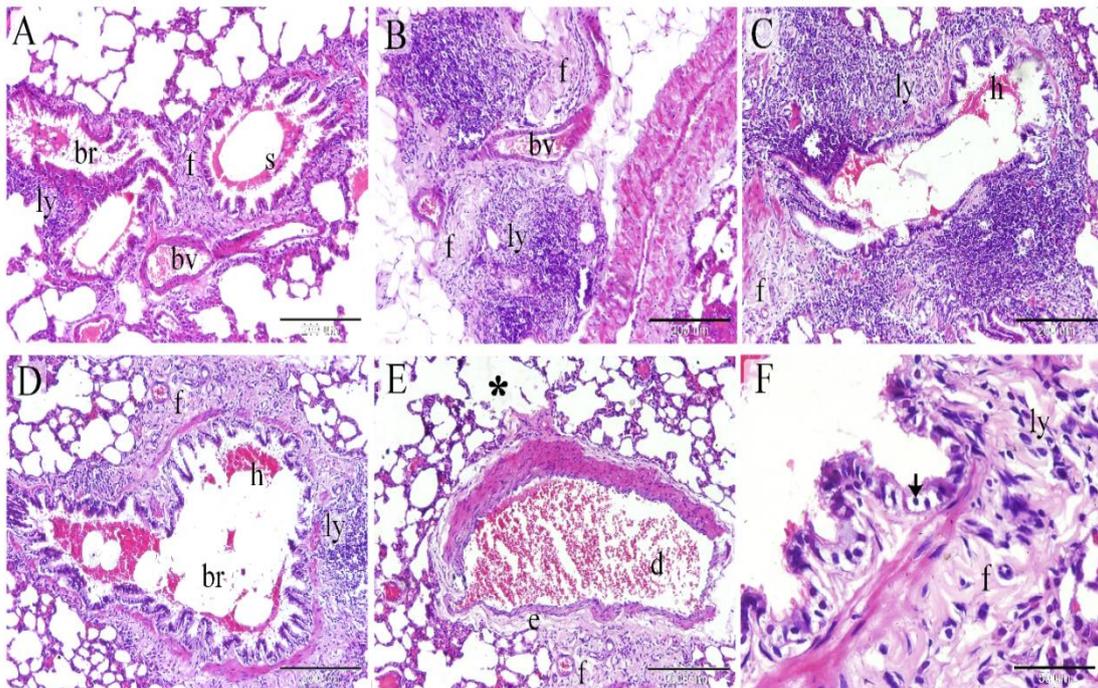


Fig. 3. Inflammatory and Fibrotic changes of the lung (pneumonia and pulmonary fibrosis). A: fibrosis (f) was detected in the interstitial tissue, bronchial epithelial sloughing (s) or desquamation was identified. *Bronchiolitis* and vasculitis was detected by diffuse inflammatory cells with infiltration around the bronchiole (br) and blood vessel (bv). B-D: multiple aggregations of inflammatory cells (ly) infiltration. Extensive fibrosis (f) was identified at the inflammatory site. Note blood vessels (bv). Intra- bronchiolar hemorrhage (h)

associated with severe *Bronchiolitis*. E: irregular dilation of the bronchioles with extensive peribronchiolar edema (e) and fibrosis (f). F: *bronchiolitis* (br) associated with epithelial apoptosis (arrow), severe infiltration of inflammatory cells (ly) and extensive fibrotic changes (f).

Follicular *peribronchiolar* and *perivascular* lymphoid hyperplasia

Follicular bronchiolitis and vasculitis were associated with nodular or follicular lymphoid hyperplasia, which was described by the formation of germinal centers in lymphoid follicles. Follicular lymphoid hyperplasia was a lymphoid proliferation

with a fibrosis reactive centre or a germinal centre with small mature lymphocytes aggregation (Fig. 4 A-I). The germinal centers of hyperplastic lymphoid follicles also showed degenerative changes in the cells. The degenerated cells were identified by *cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation* (Fig. 4 I).

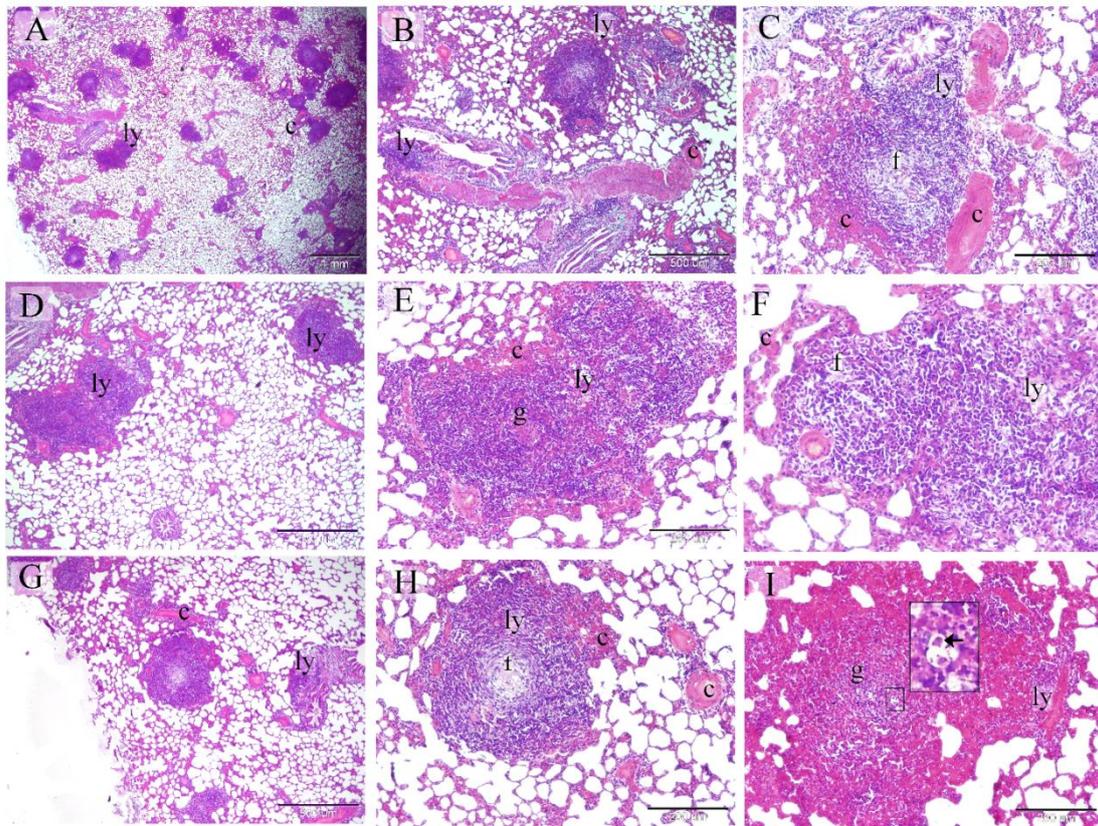


Fig. 4: Follicular *peribronchiolar* and *perivascular* lymphoid hyperplasia A-I: Follicular *bronchiolitis* and vasculitis associated with nodular or follicular lymphoid hyperplasia, which was characterized by the development of lymphoid follicles (ly) with germinal centers. The Follicular lymphoid hyperplasia was a centric lymphoid proliferation with follicular formation, which had a fibrosis reactive center (f) or a germinal center (g) with small mature lymphocytes aggregation. Note congestion (C) of the pulmonary and inter-alveolar blood capillaries. The squared area refers to degenerated cells (arrow) which identified by *cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation*.

Accumulation of hemosiderin pigment

Hemosiderin pigments deposits were recognized in the interstitial tissue around the bronchiole (Fig. 5 A), in the perivascular tissue (Fig. 5 B), in the hyperplastic *peribronchiolar* lymphoid follicle (Fig. 5 C) and intravascular (Fig. 5 D).

Alveolitis and alveolar damage

Sever alveolitis characterized by infiltration of the inflammatory and lymphoid cells in the inter-alveolar septa Inter-alveolar and intra- alveolar hemorrhage. Alveolitis was associated with bronchiolitis, which exhibited infiltration of the inflammatory cells in the bronchiolar lumen and as well as lymphatic infiltration in the bronchiolar wall (Fig. 6 A-F).

Formation of pulmonary emphysematous

Destruction of the interalveolar septum of some alveoli resulting in formation of the pulmonary emphysematous (Fig. 6 A, C, E).

Identification of the interstitial fibers using Sirius red stain

Fibrous components of the control samples of the lung were identified by Sirius red. Fibrous tissue appeared in the peribronchiolar, perivascular tissue and the alveolar septum (Fig 7 A-D).

Detection of pulmonary fibrosis using Sirius red stain

Extensive pulmonary fibrosis was recognized around the blood vessels, and in the alveolar wall (Fig 8 A-G).

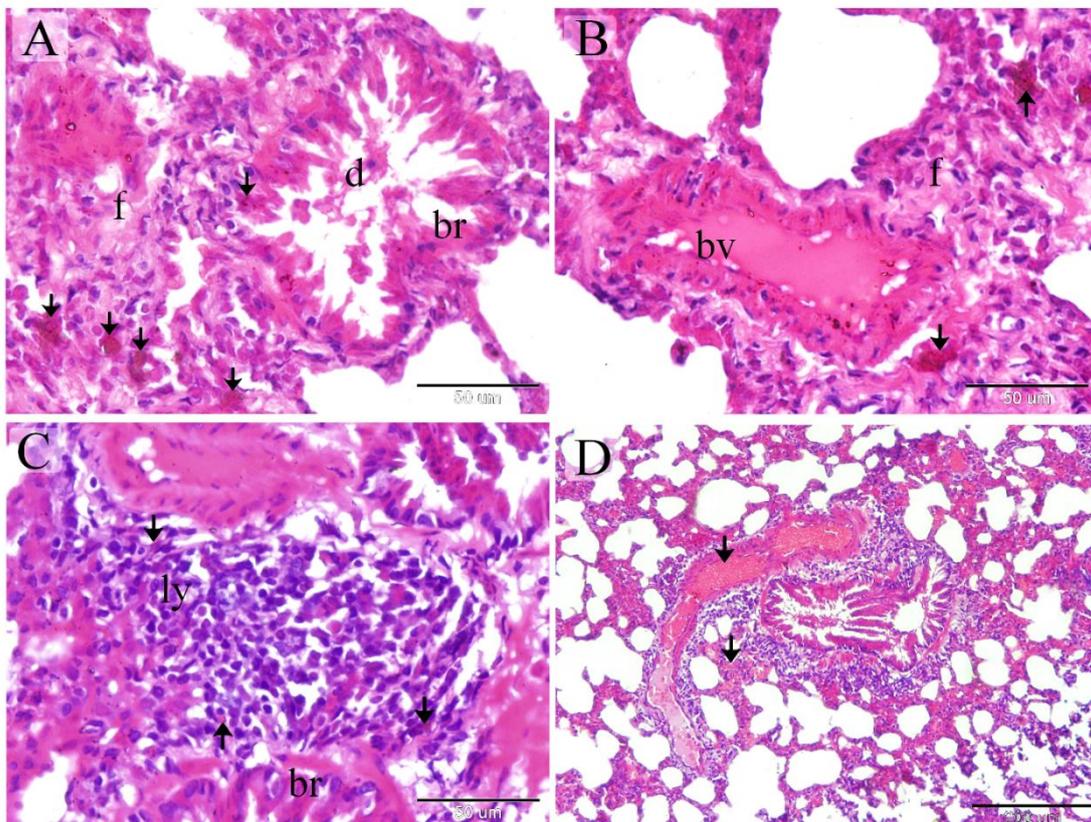


Fig. 5 Accumulation of hemosiderin pigment

A: hemosiderin pigments (arrows) were recognized in the interstitial tissue around the bronchiole (br). B: hemosiderin pigments (arrows) were identified in the perivascular tissue. Note blood vessel (bv). C: hemosiderin pigments (arrows) were distinguished in the hyperplastic *peribronchiolar* lymphoid follicle (ly). Note bronchiole (br). D: hemosiderin pigments (arrows) were distinguished in intravascular.

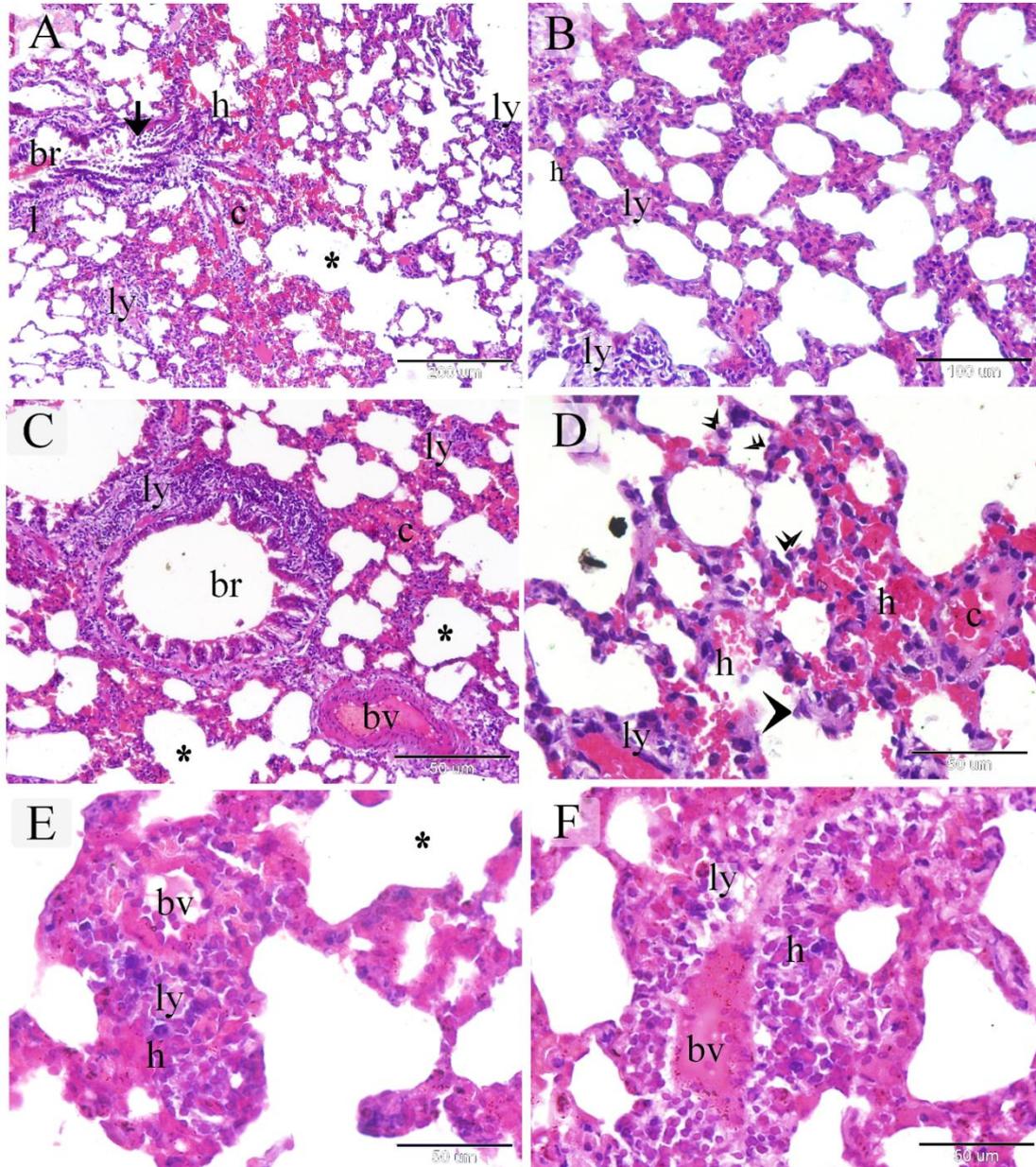


Fig. 6 Alveolitis and alveolar damage. A-D: Sever alveolitis characterized by infiltration of the inflammatory and lymphoid cells (ly) in the inter-alveolar septa Inter-alveolar and intra-alveolar hemorrhage (h). Bronchiolitis exhibited Infiltration of the inflammatory cells in the bronchiolar lumen (lumen) and as well as lymphatic infiltration (l) in the bronchiolar wall. Destruction of the interalveolar septum of some alveoli resulting in formation of the emphysematous bullae (asterisks). Some alveoli exhibited atypical *alveolar hyperplasia*

(arrowhead) which was recognized by thickening of the alveolar wall formed by multilayered pneumocytes. Focal pneumocyte hyperplasia (adenomatoid proliferation of alveolar epithelium). Cytoplasmic vacuolation of macrophages (double arrowhead) were numerous. E, F: Sever alveolitis identified by inter-alveolar lymphatic infiltration (ly) and hemorrhage (h). Note blood vessel (bv).

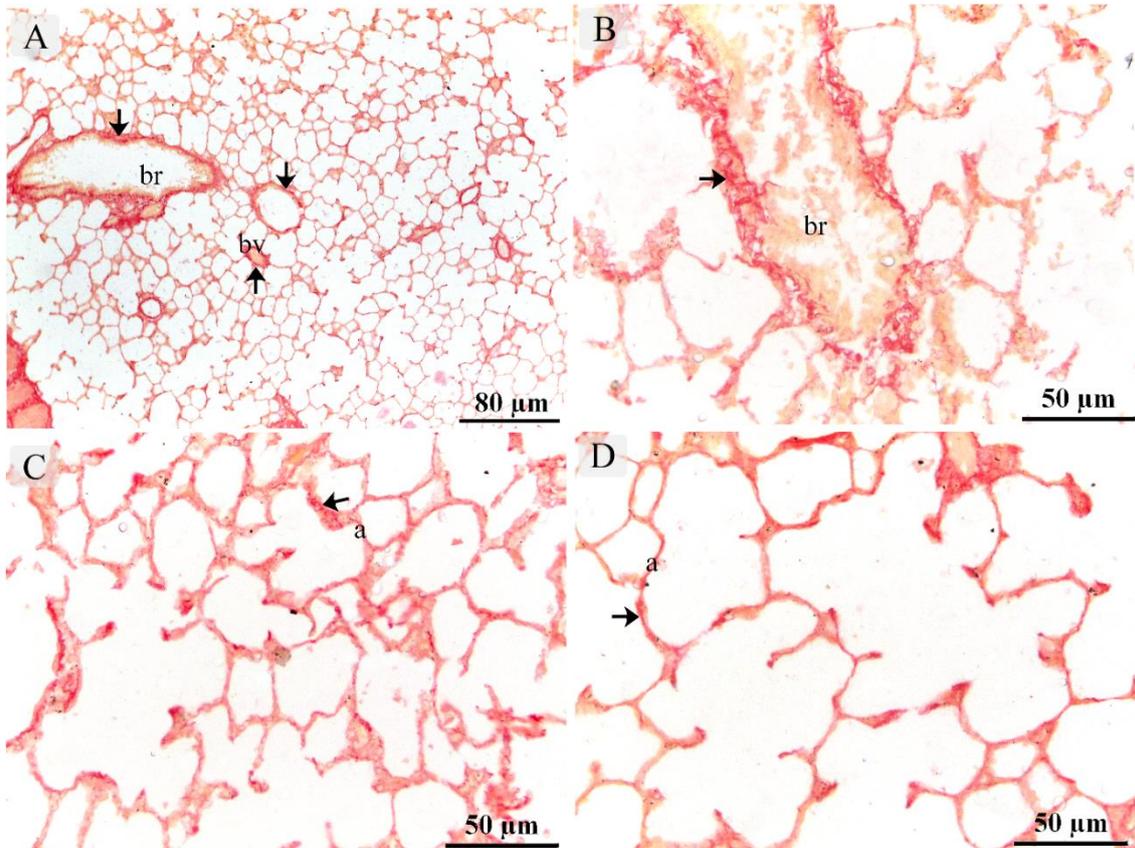


Fig 7: Identification of the interstitial fibers using Sirius red stain

Fibrous components (f) of the lung stained red by Sirius red. Note bronchiole (br), blood vessel (bv) alveolar wall (a).

Discussion

The purpose of this study was to investigate the harmful effects of formaldehyde on mice's lungs and provide clear histological evidence of those pathological changes in entire organs such as the lungs. For those who work in histology, anatomy, and histopathology laboratories around the world, exposure to numerous toxic chemicals such as formaldehyde creates a hidden health danger. In addition, formaldehyde is one of

the most commonly used compounds in those environments.

Many human studies have shown that formaldehyde exposure causes significant ocular and upper respiratory tract irritation (Weber-Tschopp et al., 1977, Kulle et al., 1987). This was also observed in the exposed animals in my study. Watery eyes (tears) in the eyes of the exposed mice indicated eye irritation, while the presence of nasal secretions in the exposed mice's nostrils indicated upper respiratory tract irritation (Arts et al., 2006). The inhibition of mucociliary function causes goblet cell

metaplasia, which has also been described by (Morgan et al., 1986b). The lymphomononuclear cells observed in the epithelium and sub epithelial tissue in this

study may be linked to reactive changes in response to frequent irritation by formalin gas. which has been confirmed in previous studies (Kerns et al., 1983).

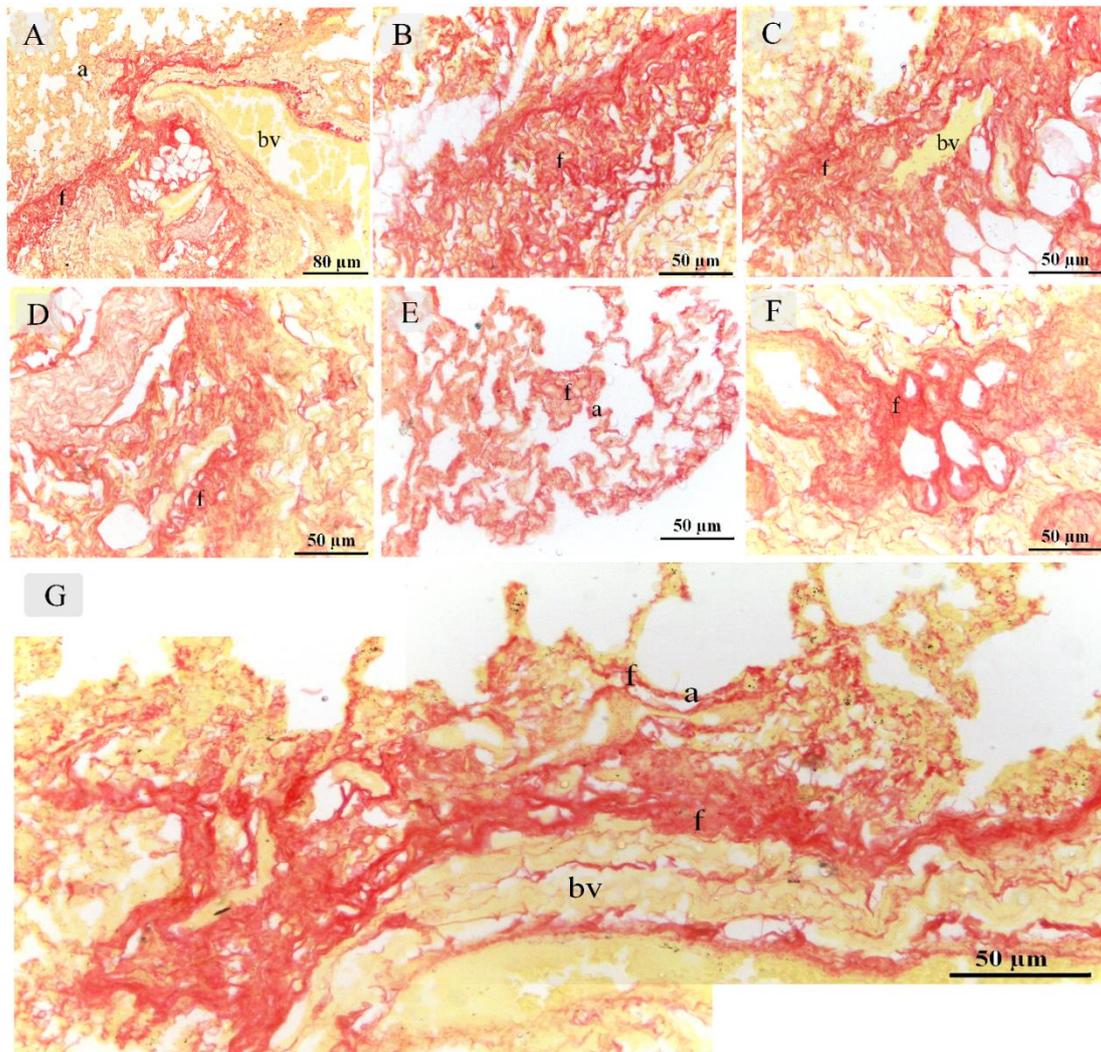


Fig 8: Detection of pulmonary fibrosis using Sirius red stain

A-G: Extensive pulmonary fibrosis (f) was recognized around the blood vessels (bv), and in the alveolar wall (a).

The results of this study showed that the histological changes in the animals exposed to 40 percent formaldehyde solution were visible and well defined.

Similarly, (Edling et al., 1988) found that a few hours of exposure to high concentrations of formaldehyde causes significant damage to the lung mucosa.

The histopathological changes indicated by (Javedan and Entezarizaher,

1999) appeared severely in rats following subacute and acute exposure to formaldehyde, similar to our observations that histological changes were more prominent in treated mice with 40 % formaldehyde solution.

Histological changes in Mice's lung tissue following formaldehyde exposure were similar to the findings described by (Casset et al., 2006). The observed changes

in the cytoarchitecture of the lungs of mice in our study suggest that formaldehyde is toxic to pulmonary cells, and those histological modifications are similar to the findings of (Odinko et al., 2012) and (McLaughlin, 1994) but disagree with that observed by (Kerns et al., 1983), that in various chronic studies, after formaldehyde exposure no histological changes or deteriorations were found in the lung tissue. As a result, the observed progressive parenchymal hemorrhage and edema which is similar to the result of (Binawara et al., 2010) , that documented formaldehyde exposure results in histopathological changes and lung tissue damage.

On the other hand , the observed cellular infiltrations and epithelial cell degenerations, confirming the inflammatory changes following formaldehyde exposure , since, according to (Njoya et al., 2009) it can cause inflammation and degenerative changes based on its natural capacity to stimulate epithelial degeneration.

Because of its volatility, formaldehyde is toxic at certain concentrations and its harmful effects are increased at room temperature. All who work closely with formaldehyde, such as embalmers, anatomists, technicians, and medical or veterinary students, must be aware of its danger. The current study found that exposure to concentrated formaldehyde for 5 hours per day for 2 months had an irritating effect, particularly on the respiratory organs (ex. lungs); this findings are consistent with (Njoya et al., 2009), who found that formaldehyde's primary target is the respiratory system.

Inhalation of formaldehyde at appropriate exposure concentrations causes severe damage in certain regions of epithelial tissue in the upper respiratory tract of rats, mice, and monkeys, according to animal studies(Chang et al., 1983)

,(Monticello et al., 1989) ,(Monticello et al., 1991) and (Morgan et al., 1986a).

Many pulmonary histological changes appeared after formaldehyde exposure, including dysplasia, metaplasia, mononuclear inflammatory cells infiltration, epithelial desquamation, alveolar septal destruction, congestion of blood vessels, and interstitial fibrosis. These findings are in agreement with a number of previous studies at different doses of formaldehyde exposure, such as (Monticello et al., 1989, Kane and Alarie, 1977) .

Changes in polymorphonuclear cells, which are known to be the first line of defense against bacterial infections, those changes make it responsible for the lower and upper respiratory tract's reduced resistance after formaldehyde exposure (Chambers et al., 2018) , and those results are in line with our study ,As the observations showed reduced immunity of mice and sever weight loss.

Those changes, which were characterized by, ulceration in the alveoli, hyperkeratosis and necrotic lesions, definitely will lead to bronchial epithelial sloughing and desquamation. Subsequently this might affect the functional capacity of the lung. Similarly (Njoya et al., 2009, Bansal et al., 2011) recorded the same histopathological changes in their results on adult wistar rats and respiratory organs of rabbits .

Accumulation of hemosiderin pigment, which were recognized in the interstitial tissue around the bronchioles, in the perivascular and intravascular tissue .These hemosiderin depositions are most probably due to extravasations of blood, hemorrhage or congestion following chronic exposure to formaldehyde. Those findings are similar to that recorded in many previous toxicity studies and histopathological findings in

cynomolgus monkeys confirmed by (Sato et al., 2012) .

Conclusion

In conclusion, the current study's findings clearly illustrated that formaldehyde exposure causes an increase in the number of apoptotic cells in lung tissue and BALT (bronchial associated lymphoid tissue).

As a result, we recommend that further efforts should be taken to replace formaldehyde with less hazardous chemical, as well as educating embalming laboratory users, technicians, medical and veterinary students of the formaldehyde vapor hazards linked with the impeding technique.

Conflict of interest

The authors declare that there is no conflict of interest.

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