HISTOPATHOLOGIC CHANGES IN RAT ORGANS UPON CHRONIC EXPOSURE TO FORMALDEHYDE VAPOR

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ABSTRACT

The present study was carried out to detect histopathologic lesions in different rat organs after chronic exposure to formaldehyde vapor. Forty adult albino Wistar rats were used in this experiment. Animals were divided into two groups; Control group (n=10; 5 males and 5 females) which were not exposed to formaldehyde at all, while the remainders regarded as the experimental group (exposed to formaldehyde for 21 days, n=30; 15 males and 15 females). The histopathologic examination in our study revealed several changes in exposed group such as; chronic tracheitis, squamous metaplasia of lining epithelium and tracheal glands with occurrence dysplasia in the lining epithelium, interstitial pneumonia, hydropic degeneration in the hepatocytes, epidermal hyperplasia, interstitial glomerulonephritis with atrophy of glomeruli. We concluded that the overexposure of formaldehyde produced lesions in different organs but their toxic effects were seen predominantly in respiratory system.

INTRODUCTION

Formaldehyde is a flammable, colorless substance, which is readily polymerized as a gas at normal room temperature.[1] Formaldehyde is water-soluble, whose pure form is irritant and its solid state called Para formaldehyde. Formaldehyde concentration is generally explained as parts per million (ppm; 1 ppm¼ 1.25 mg/m^3), and 40%–50% of its aqueous solution is called formalin.[2] Because of the extensive uses of formaldehyde in building materials, textiles, insulation and other industries, there is a potential for occupational and environmental exposure. Considerable human exposure to formaldehyde gas occurs at concentrations up to 1 ppm.[3]

Formaldehyde is readily absorbed from the respiratory tract following inhalation and from the gastrointestinal tract following ingestion, but is poorly absorbed following dermal exposure.[4] The predominant effects following an acute inhalation exposure to formaldehyde is irritation and burning of the mucous membranes of the nose, mouth and upper respiratory tract. Some adverse effects following acute exposure to large amounts of formaldehyde may include weakness,

headache, nausea, vomiting, pneumonia, dyspnea, wheezing, coughing, laryngeal and pulmonary edema, bronchospasm, laryngeal spasm, respiratory depression, obstructive tracheo-bronchitis, central nervous system depression, convulsions and coma.[5]

Although formaldehyde has recently been classified by the International Agency for Research on Cancer (IARC) as "carcinogenic in humans" (class 1), it is still widely used in anatomy and pathology departments for fixation and preservation of biological tissues. Its use, therefore, raises the question of occupational exposure.[6]

In rats which were exposed to 10 ppm formaldehyde inhalation 8 hours/day, 5 days/week for 8 weeks, the lining epithelium of respiratory mucosa showed a loss of ciliated cells with metaplasia of goblet cells.[7] Rats inhaled 20.3 ppm formaldehyde gas for 13 weeks, 8 hours/day, 5 days a week, induced a variety of toxic effects in the liver tissue such as hepatic enlargement, hepato-cellular fatty degeneration and hepatic necrosis.[8] An experiment study on rat kidney showed that rat exposure to formaldehyde vapor in the concentration of 1.5 ppm 4 hours/day, for 4 days/week for 18 weeks led to mild congestion in the glomeruli, focal congestion and vacuolar (hydropic) degeneration of tubular cells, and necrosis.[2] The aim of the present study was to investigate histopathologic lesions in different rat organs after chronic exposure of formaldehyde vapor.

MATERIALS AND METHODS

Study area:

This study was conducted from June 1, 2013 to September 1, 2013 into two different locations; the first part of the study which dealt with housing and exposing rat to formaldehyde vapor in the animal house of College of Medicine/Hawler Medical University; the second part which included scarifying and taking biopsy from tissues (liver, trachea, lung, kidney and skin) which were then stained with Haematoxylin and Eosin (H&E) which was performed in the Research Pathology Lab/School of Medicine Slemani University.

Animal model:

Forty adult albino Wistar rats were used in this experiment, each of which weighing 200-300gms, fed with standard pellet diet (Pico Lab) and provided water *ad libitum*. Animals were housed under a controlled room temperature of about 22-25 ºC and photoperiodicity of the 12 hours day/12 hours night.

Experiment design

After obtaining the approval of ethical committee, the animals were assigned into two groups; Group A, which were not exposed to Formaldehyde regarded as control group (n=10; 5 males and 5 males) and group B, which were regarded as exposure group (exposed to formaldehyde, n=30; 15 males and 15 females). Animals were exposed to 40% formaldehyde for 5 hours/day for 21 days consecutively. Two special cabinets which were manufactured from PVC and glass windows (dimensions 60 x 60 x 60 cm) were used in this experiment. Twenty ml of formaldehyde were placed in a petri-dish and elevated 30 cm from the cabinet ground to avoid drinking of solvent during exposure.

Sampling method:-

At the end of the experiment, the animals were euthanized using ketamine hydrochloride (100 mg/Kg) and xylazine. Tissue samples were taken from skin, trachea, lung, kidney and liver. Specimens were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed. The tissues were paraffin embedded and sections of 5µm thickness were obtained and stained with haematoxylin and eosin to detect any abnormal lesions formed by formaldehyde exposure.

RESULTS

For better understanding this study, the results were separated according to the type of the specimens into the following:

Trachea

The histologic sections of the trachea in the control group showed normal mucosa which composed of respiratory epithelium (ciliated-pseudostratified columnar epithelium with Goblet cells), a thin layer of loose connective tissue (lamina propria) and muscularis mucosa (Figure 1), while in exposed group reveled several pathologic changes were observed as focal dysplasia, squamous metaplasia of lining epithelium (respiratory epithelium replaced by stratified squamous epithelium), loss of cilia and Goblet cells (Figure 2), sub-epithelial infiltration of mononuclear inflammatory cells predominantly plasma cells with few lymphocytes and histiocytes. Tracheal glands became larger in size due to hyperplasia of glandular epithelium; the lumen of the glands were dilated and some contained inflammatory cells (Figure 3).

Lung

In the histological section of the lung in the control group the normal architecture of the lung was preserved (Figure 5). While in the exposed group lung showed infiltration of mononuclear inflammatory cells in the interlobular septa with a large number of RBCs (hemorrhage), destruction of alveolar septa, congestion of blood vessels, moderate to extensive fibrosis in the interstitial tissue (Figure 4), irregular dilation of the bronchioles with dense peri-bronchiolar lymphocytic infiltration, Goblet cells hyperplasia, and the bronchiolar lumen contains a large number of desquamated epithelium (Figure 6).

Liver

In the histological section of liver in control group no changes were observed (Figure 7), when compared to the liver of exposed group showed dilation of central vein, enlargement of sinusoids, cloudy swelling of hepatocytes (multiple opaque vacuoles present within the cytoplasm with centrally located nuclei) as shown in figure 8.

Kidney

The histological section of kidney showed normal structural appearance in control group (Figure 9), whereas in exposure group showed atrophy of glomleruli, degeneration of convoluted tubules, interstitial infiltration of small mature lymphocytes (Figure 10) and focal areas of increased glomerular cellularity in the form of mesangial expansion and diffuse thickening of the glomerular capillary walls with multifocal vascular congestion (Figure 11).

Skin

Histological section of the skin in exposure group showed focal thickening of the epidermis (epidermal hyperplasia) due to increasing cell numbers in the stratum spinosum and stratum granulosum. No microscopic changes were observed in the

dermis, whereas the skin of the control group showed normal thin epidermis, dense collagenous dermis and skin appendages (Figure 12).

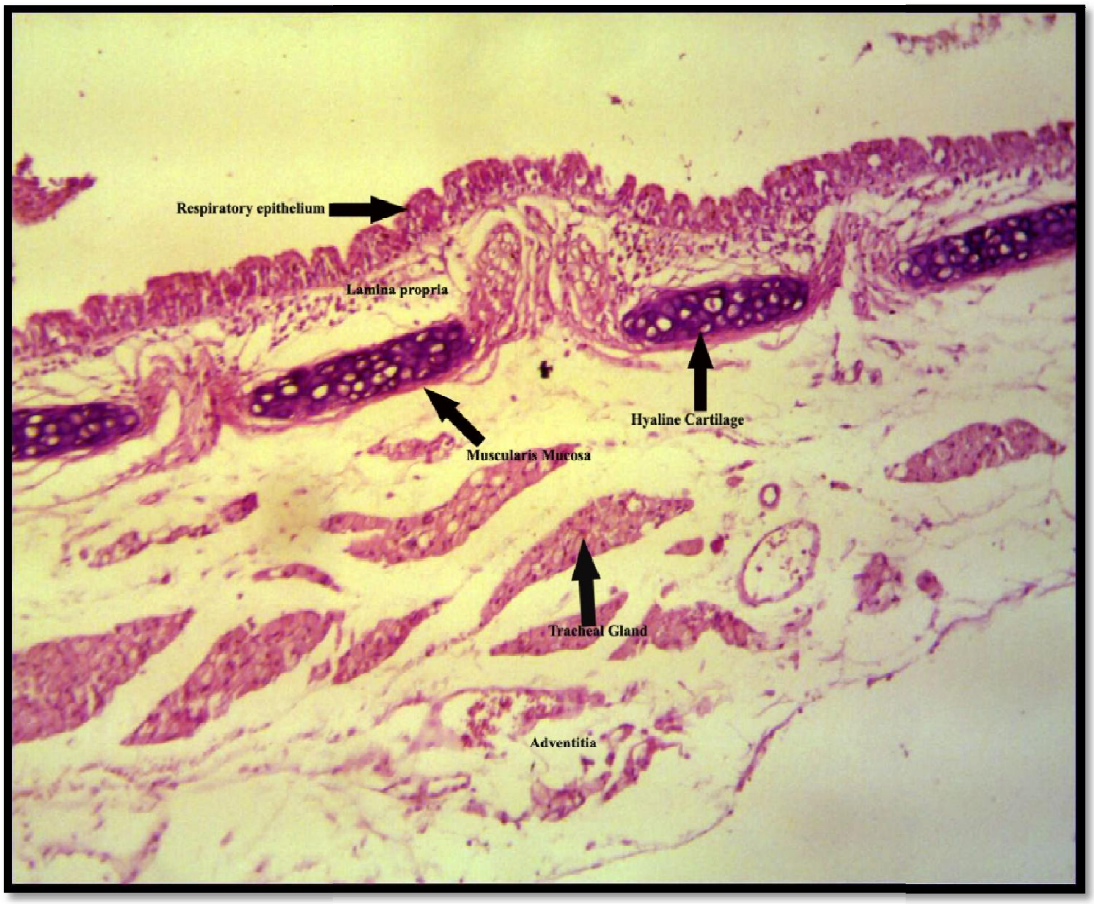

Figure 1. Normal histological view of rat trachea in the control group (H&E stain, X100).

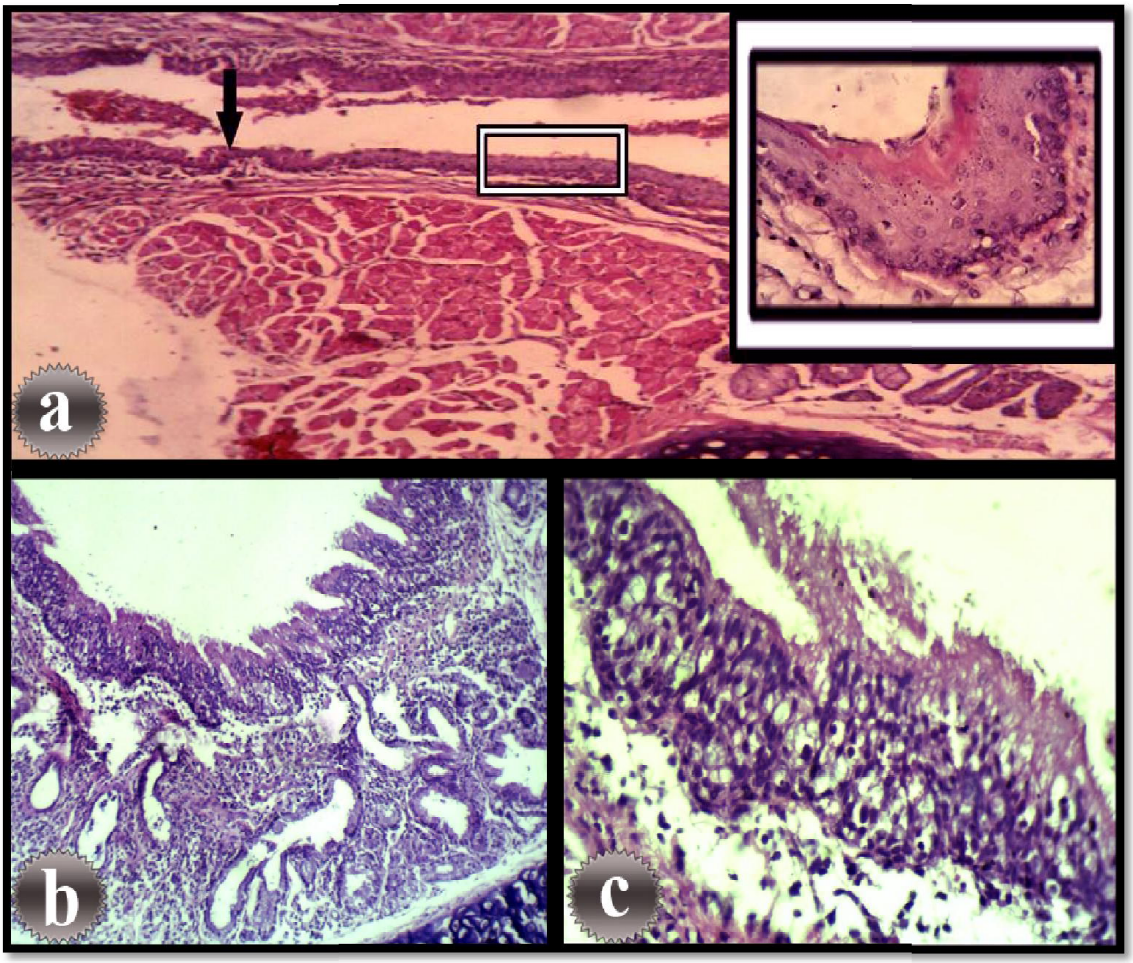

Figure 2. A: A transition area (black arrow) from ciliated-pseudostratified columnar epithelium to stratified squamous epithelium. Two rectangular areas revealed a specific section in the tracheal epithelium changed to stratified squamous epithelium after magnification (Inset) (H&E stains, X100, X400), B and C: Epithelial dysplasia (H& E stain, X100, X400).

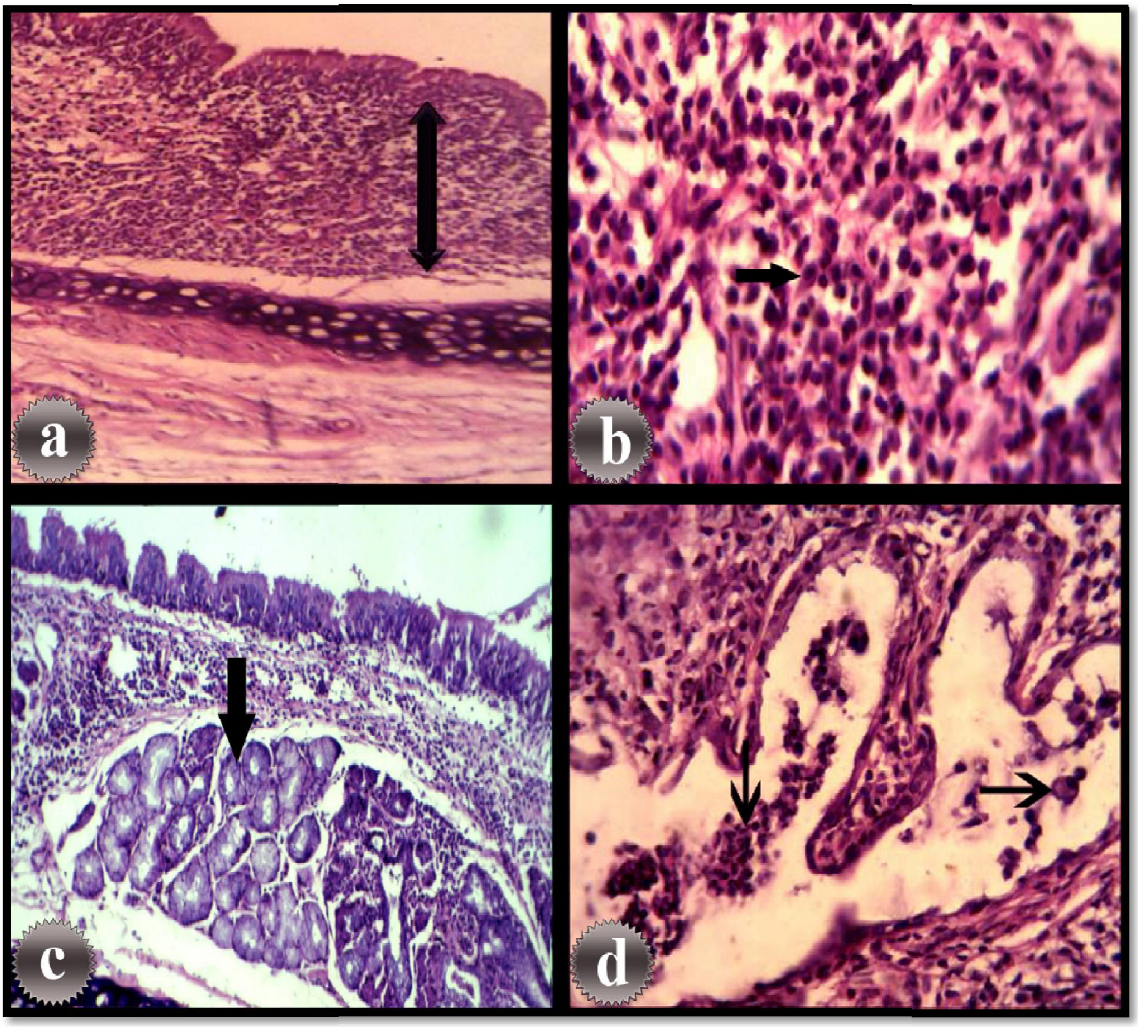

Figure 3. A: Sub-epithelial infiltration of mononuclear inflammatory cells as indicated by black double-headed arrow (H&E stain, X40), B: Infiltration of large number of mononuclear inflammatory cells (Black arrow, H&E stain, X400). C: Increasing the number of the tracheal glandular epithelium (Black arrow, H&E stain, X100), D: Dilation of tracheal gland and intraluminal accumulation of inflammatory cells (Black arrows, H&E stain, X400).

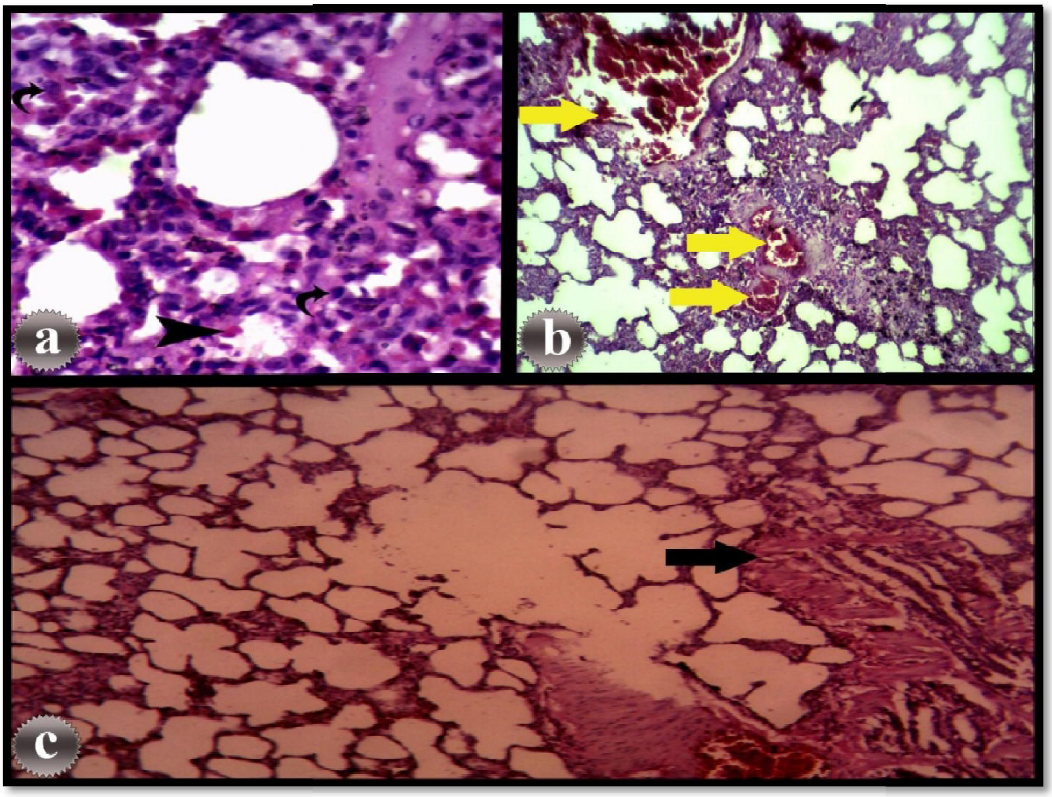

Figure 4. A: Infiltration of mononuclear inflammatory cells in the interlobular septa (Curved arrows) with a large number of RBCs as indicated by an arrow head (H&E stains, X400), B: Congestion of blood vessels as indicated by arrows (H&E stain, X100) and C: Destruction of alveolar septa and fibrosis in the interstitial tissue as indicated by black arrow (H&E stain, X400).

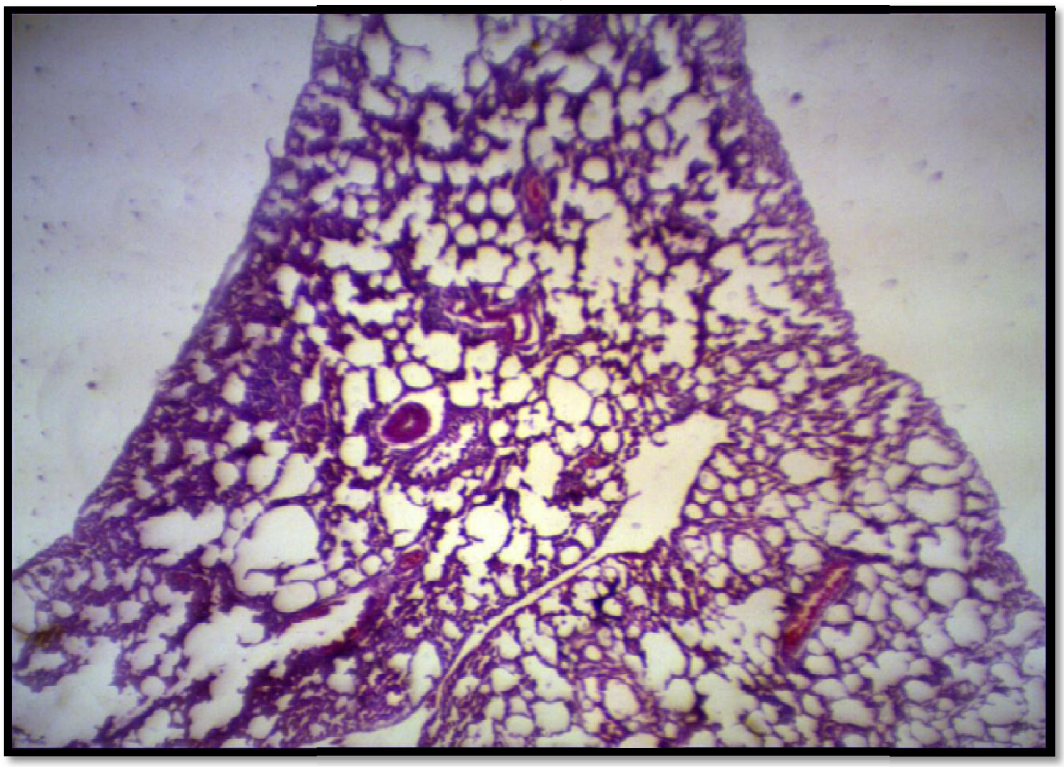

Figure 5. Normal histological section view of rat lung in the control group (H&E stain, X100).

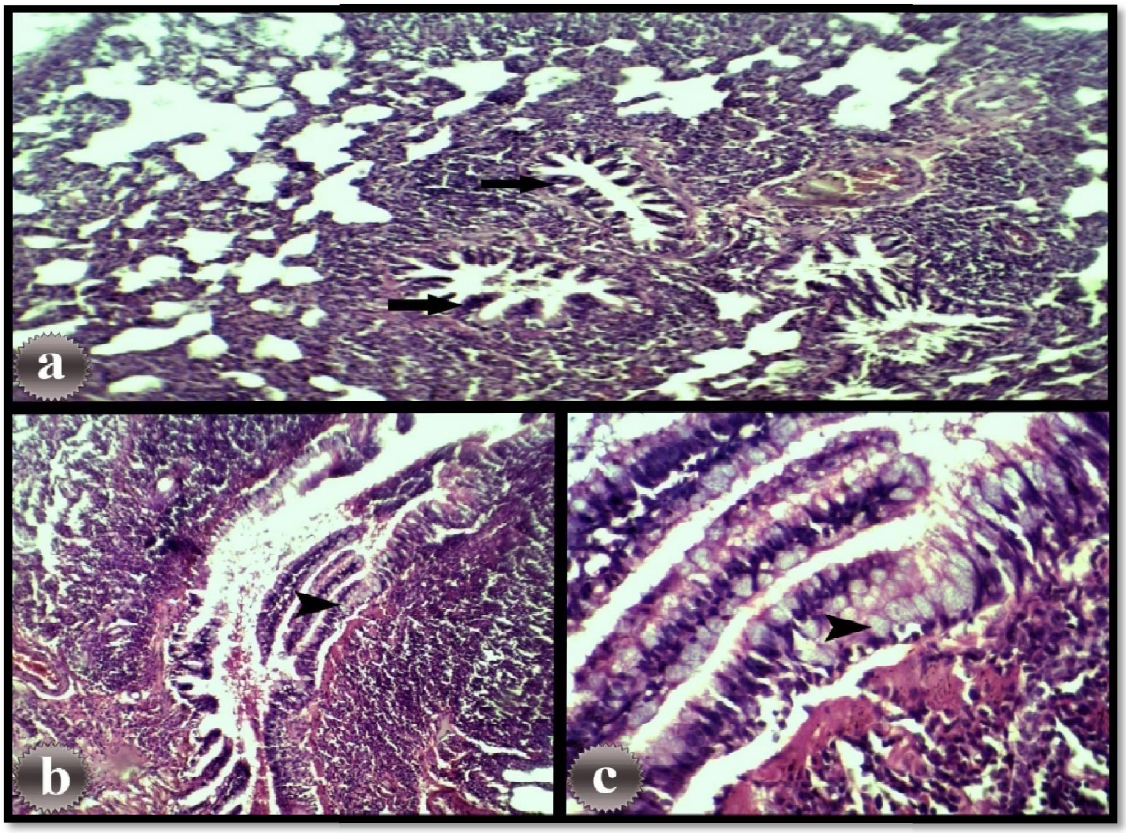

Figure 6. Lung section show A: Peri-bronchiolar lymphocytic infiltration of terminal bronchioles (Black arrows, H&E stain, X40), B and C: Irregular dilation of bronchi with goblet cell hyperplasia in the lining of the bronchi as shown by black head arrows (H&E stain, X100, X400).

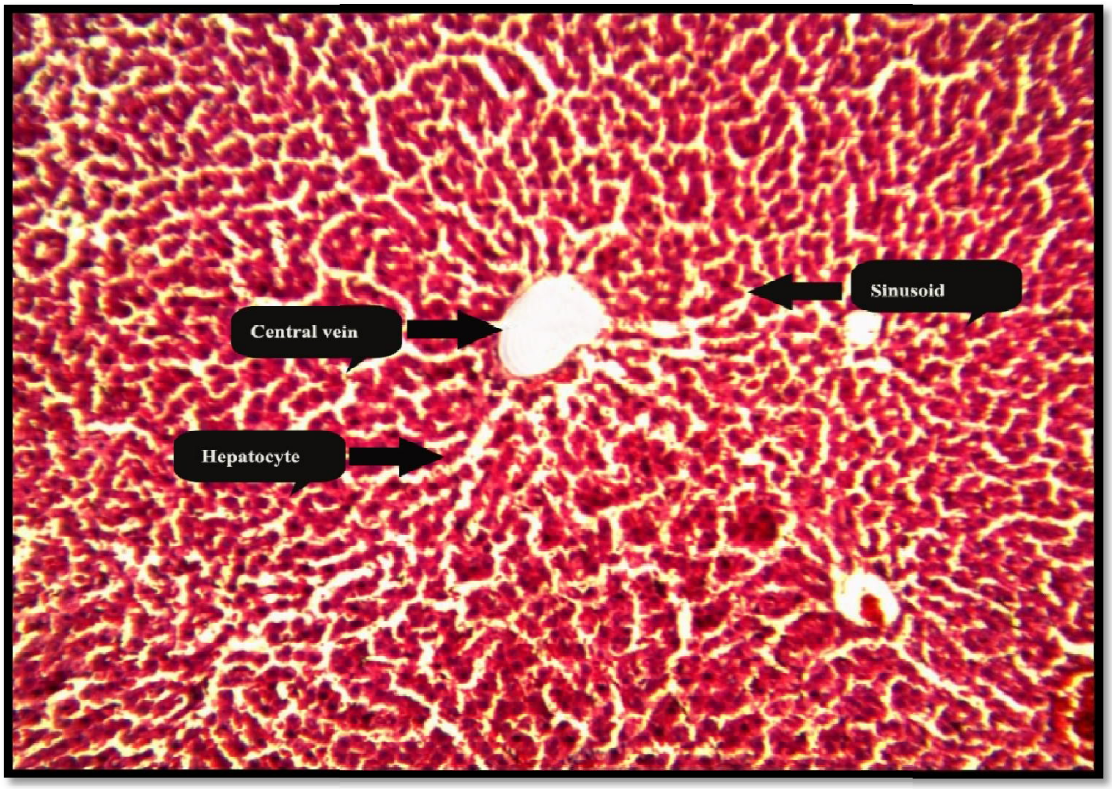

Figure 7. Normal histological section view of rat liver in the control group (H&E stain, X100).

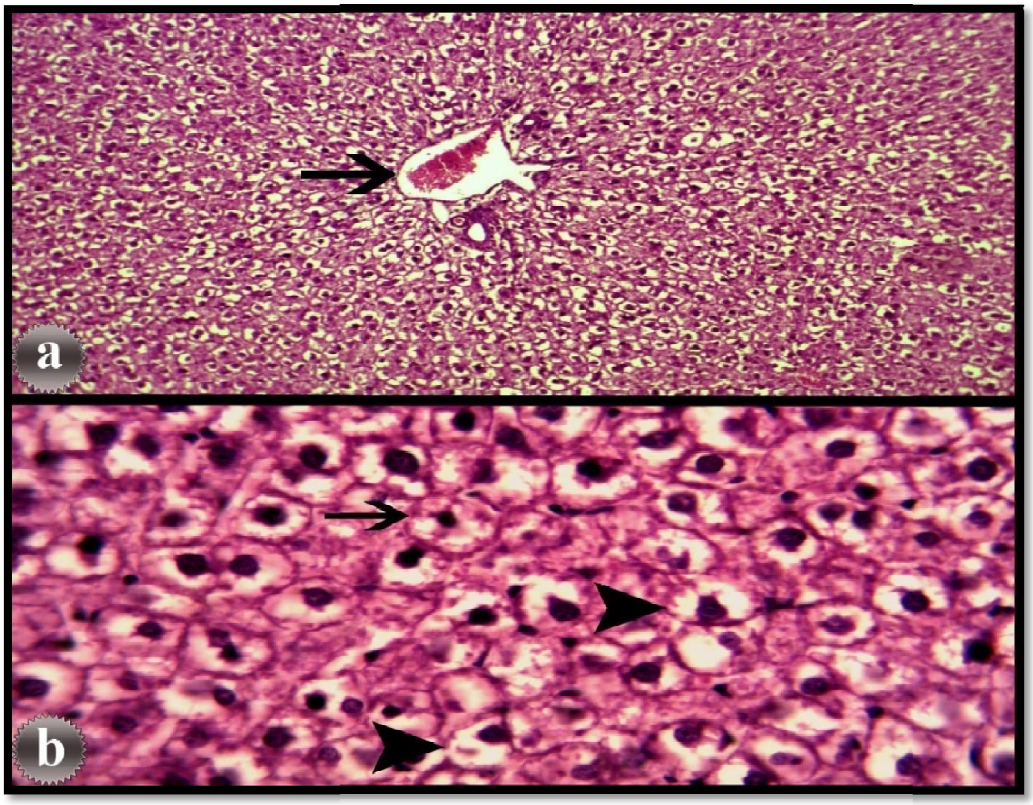

Figure 8. Liver section show A: Liver section shows a central vein dilated as indicated by black arrow (H&E stain, X200), B: Hepatocytes with opaque cytoplasm and centrally located nuclei regarded as cloudy swelling (Black arrow), (H&E stain, X400).

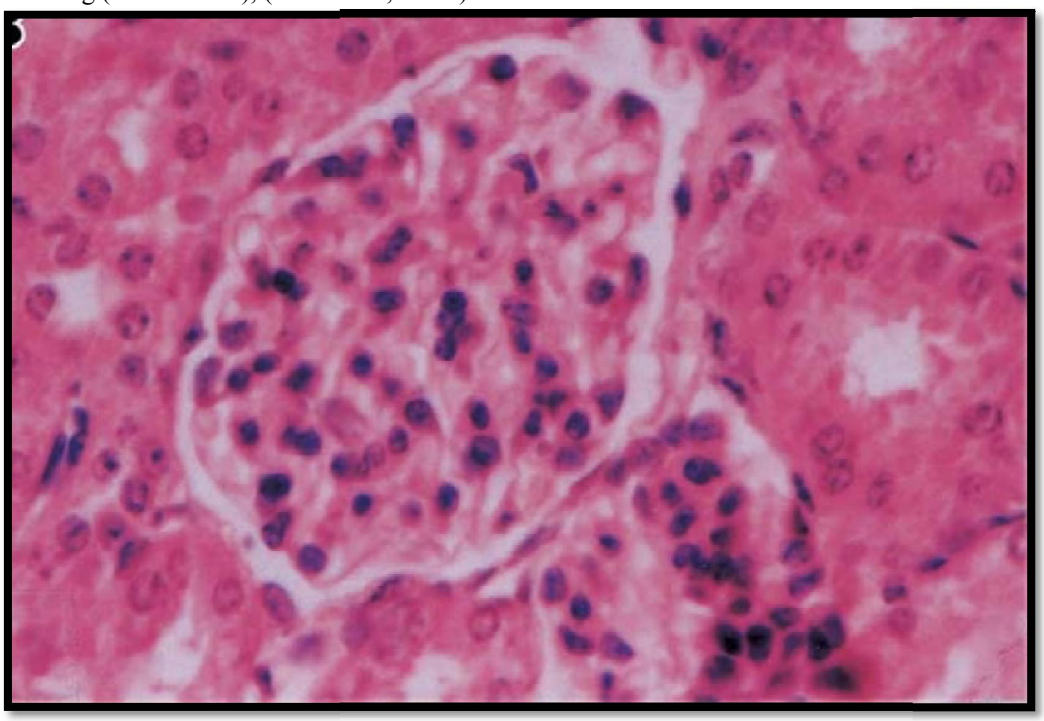

Figure 9. Normal histological section view of rat kidney in the control group (H&E stain, X100).

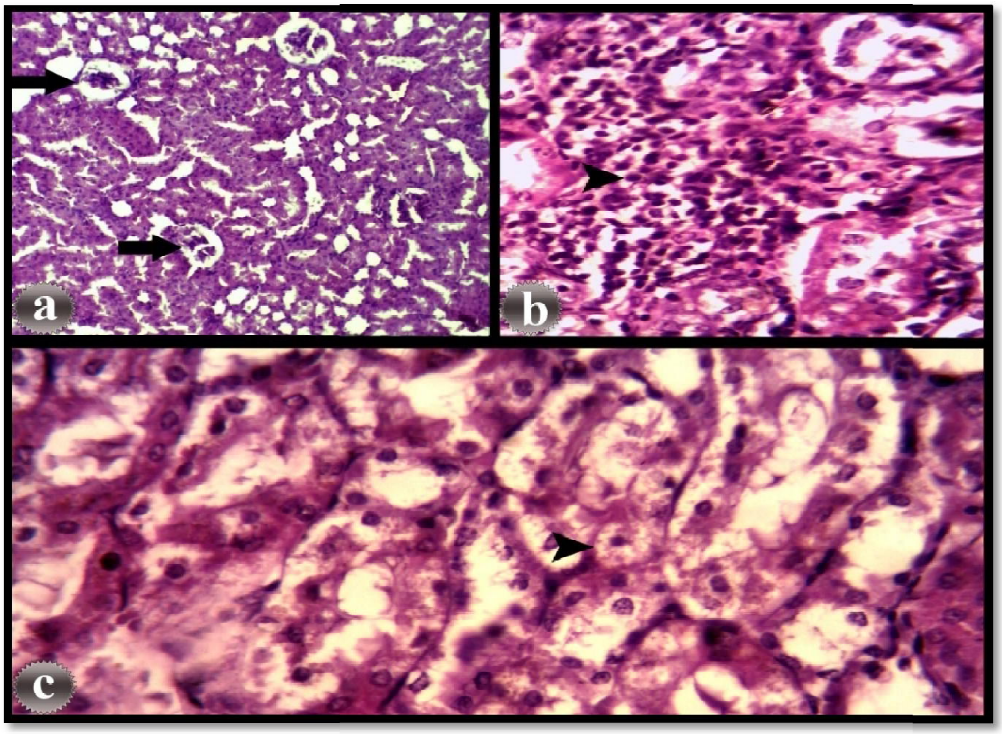

Figure 10. Kidney section show A: Glomerular collapse or atrophy (Black arrows, H&E stain, X40), B: Infiltration of lymphocytes in the interstitial tissue (Arrow head, H&E stain, X400), C: Cloudy swelling in the epithelial lining of the renal tubules as indicated by an arrow head (H&E stain, X400).

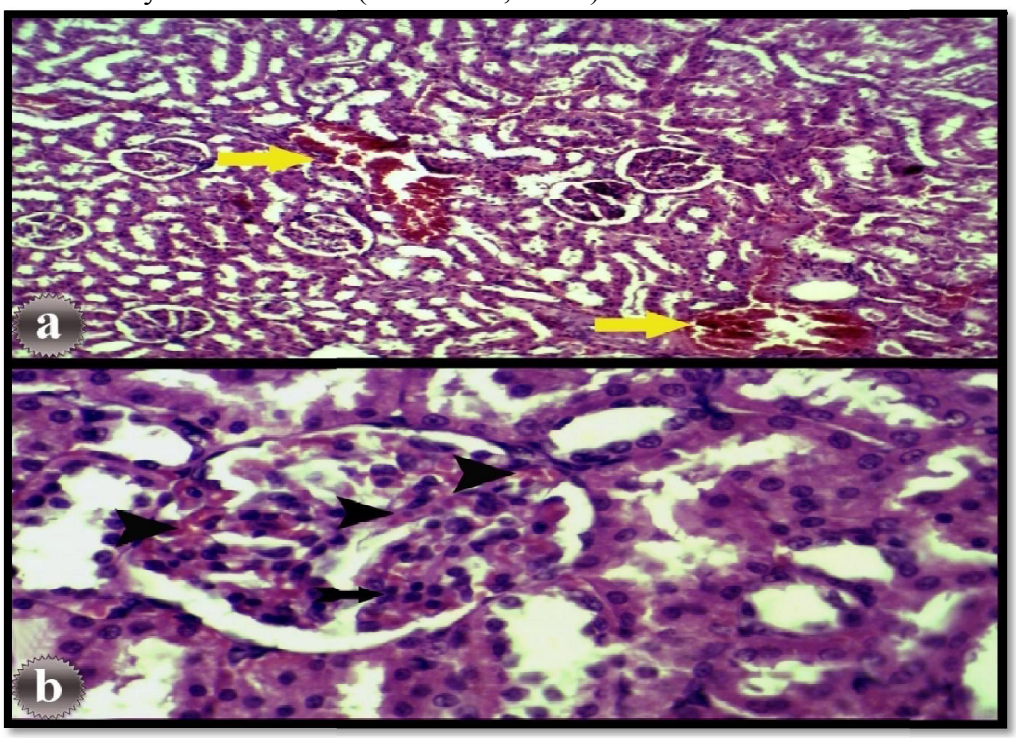

Figure 11. Kidney section show A: Vascular congestion as indicated by yellow arrows (H&E stain, X100), B: Increasing glomerular cellularity in the form of mesangial expansion (Black arrow) and diffuse thickening of the glomerular capillary walls as indicated by an arrow head (H&E stain, X400).

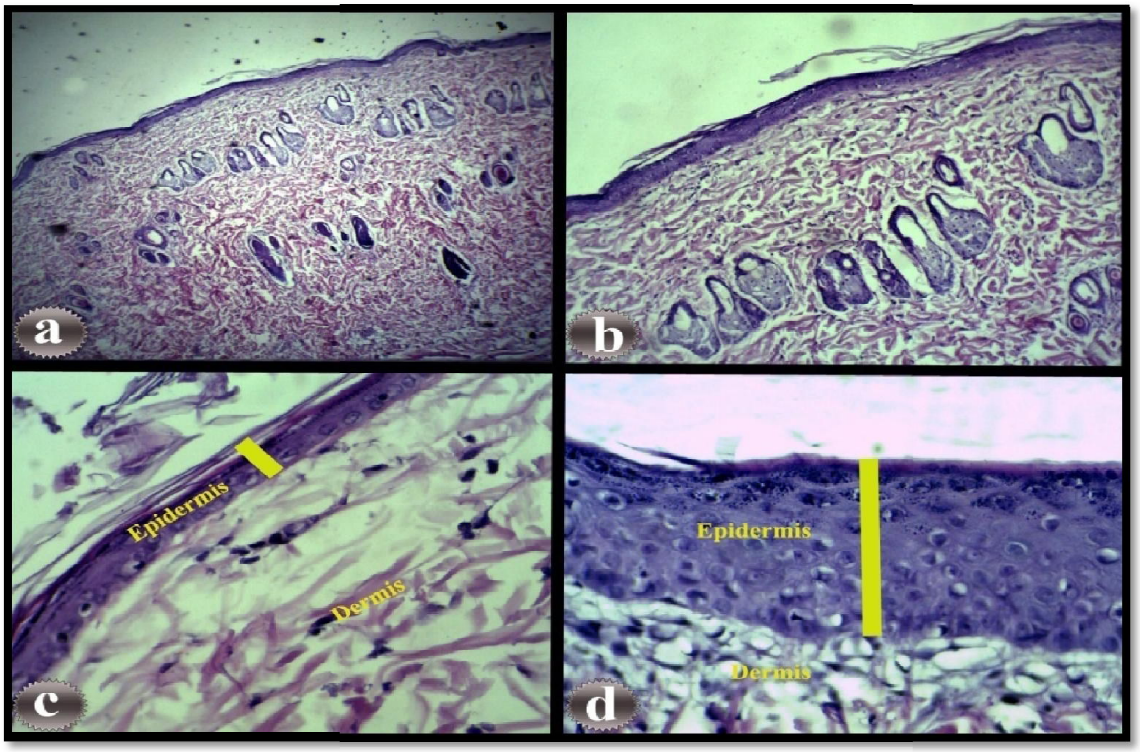

Figure 12. A-C: Normal histological section view of rat skin in the control group (H&E stains, X40, X100 and X400 respectively), and D: Focal epidermal hyperplasia in the exposed group (H&E stain, X400).

DISCUSSION

Formaldehyde is recognized as toxic at certain doses and the chances of harmful effects are increased at room temperature due to its volatility.[9] The toxicity of formaldehyde is of concern to all who work closely with it. Embalmers, anatomists, technicians and medical or veterinary students are among the people who have high exposure to formaldehyde.[10]

The current study investigated that exposure to concentrated formaldehyde for 5hours/day (15 days) had an irritating effect especially on the respiratory organs (lung and trachea); this finding is in agreement with Cui et al,[11] who mentioned that the respiratory system is the major target of formaldehyde. The tracheal and pulmonary changes in this study as dysplasia, metaplasia, infiltration of mononuclear inflammatory cells and tracheal gland hyperplasia, desquamated epithelium, destruction of alveolar septa, congestion of blood vessels and interstitial fibrosis are consistent with a number of previous studies at a variable dosage of formaldehyde, period of exposure and the observations were; two previous studies on respiratory mucosa reported loss of the cilia, squamous metaplasia and infiltration of mononuclear inflammatory cells in Wistar rats,[12,13] congestion and squamous metaplasia were observed by Rusch et al,[14] in the nasal turbinate of monkey and Fischer rat. Bansal et al,[15] showed mononuclear cellular infiltrations, emphysema, loss of mucosal folds and hyperplasia of cells in the bronchiole, peribronchial lymphomononuclear cellular infiltration and congestion and haemorrhages in the respiratory organs of rabbit after exposure to formaldehyde vapor. Also Mohamed et al,[16] reported squamous cell metaplasia of trachea, thickening alveolar septum, bronchiolar epithelial hyperplasia, proliferative capillary, pulmonary vasculitis, hyperplastic parabronchiloar lymphocytic aggregations, pulmonary fibrosis and

precancerous changes (goblet cell metaplasia and bronchiolar epithelial dysplasia). A previous study on human nasal mucosa with occupational exposure to formaldehyde revealed a similar lesion of above studies such as loss of cilia, Goblet cell hyperplasia, squamous metaplasia and mild dysplasia.[17]

The present study showed that exposure to formaldehyde induced few histopathologic changes in the rat livers. These lesions included dilation of intralobular central vein, enlargement of sinusoids, cloudy swelling and fatty change which are in agreement with two previous studies. [8,18] Who observed dilation of intralobular central veins and sinusoids with mononuclear cell infiltrations in the portal areas in rat livers. In contrast to our results Woutersen et al,[19] found no microscopic lesions in liver specimens of rats exposed to 20 ppm (6 hours/day; 5 days/week for 13 weeks).

Our study induced hisopathologic changes in the kidney, which included; interstitial glomerulonephritis, congestion, cloudy swelling in the tubular cells with atrophy of glomeruli. These findings are partially in agreement with Golalipour et al,[2] who found the same lesions except the infiltration of mononuclear inflammatory cells. Several studies reported that exposure to formaldehyde vapor <10ppm showed no histopathologic lesions in rat kidney.[14,19-22]

This study showed focal thickening of the epidermis (focal hyperplasia) due to increasing cell numbers in the stratum spinosum and stratum granulosum (two cases out of thirty), while the remainders appeared as normal. No microscopic changes were found within the dermis and this finding is partially in agreement with previous studies, which they reported that formaldehyde is poorly absorbed following dermal exposure but it is corrosive and can cause irritation and burns to the skin. Repeated or prolonged dermal exposure to splashes of solutions containing formaldehyde can lead to skin irritation and may also cause skin sensitisation (allergic contact dermatitis).[23-25]

Lyapina et al,[26] determined that a number of factors affect skin absorption of formaldehyde; existing dermatitis or acne and/or if the skin is irritated when absorption is increased. High humidity of the air and the area of skin exposed also affect skin absorption of formaldehyde. Absorption appears to be limited to cell layers immediately adjacent to the point of contact. Evidence for toxic effects at distant sites is less consistent.

Hairless mice dermally exposed to 0.2 ml of a 10% aqueous solution of formaldehyde, 2 times/week for 60 weeks, developed epidermal hyperplasia and some mice developed cutaneous ulcers.[27]

Conclusion:

According to the current study, the formaldehyde inhalation with mentioned concentration and duration can induce several histopathologic changes in variety of organs in albino rat. It seems that the respiratory organs are more susceptible, kidney and liver in between and the less susceptible organ is the skin for formaldehyde vapor.

التغيرات النسيجية في أعضاء الجرذان عند التعرض لبخار الفورمالدهايد

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الخلاصة

الهدف من هذه الدراسة الكشف عن الآفة النسيجية في الأعضاء الجرذان مختلفة بعد التعرض لبخار الفورمالدهايد. استخدم فى هذه التجربة أربعون جرذاٍ بالغ من نوع ويستار (٢٠ ذكر و ٢٠ أنثى). تم تقسيم الحيوانات إلى مجموعتين؛ مجموعة السيطرة (٥ذكر و ٥ أنثى) التي لم تتعرض إلى الفورمالديهايد، في حين أن المتبقي تعتبر المجموعة التجريبية (ثلاثون:١٥ذكر و ١٥ أنثى -يتعرضون لبخار الفورمالديهايد). لمدة ٥ساعات يومياُ/٢١ يوم. الفحص النسيجي في دراستنا كشفت تغيرات مختلفة في المجموعة المعرضة فيها؛ الالتهاب الخلالي المزمن في الرئة، الالتهاب القصبات المزمن، وعسر وحؤول شائك في ظهارة بطانة المجارى التنفسية مع تغييرات حؤولي في غدد القصبة الهوائية، تنكس خزبي في خلايا الكبد، وتضخم من بشرة الجلد، التهاب كبيبات الكلى الخلالي مع ضمور الكبيبات.

استنتج من هذه الدراسة أن التعرض لبخار الفورمالديهايد ينتج آفات في مختلف الأعضاء ولكن شوهدت آثارها السامة في الغالب في الجهاز التنفسي.

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