

Histological Study of Halothane Effects on Lung in the Male White Mice

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Abstract:

In order to study the side effect of halothane on the lung in the male white mice, the present work carried out (80) male white mice, which were divided into three groups. Each group was treated with halothane per 15 minutes through thirty days. The study designated to estimate the histological changes in three treated groups after exposure to three different concentrations of halothane in lung. The tissue section of lung that exposure to 1% concentration of halothane have some alveoli destruction and aggregation of inflammatory cells around the cystic dilation, so showed small aggregation of pulmonary alveolar macrophage (PAM) in some of cystic dilation. The current results showed the third zones of bronchial tree filled with secretion, prominent emphysema, with proliferation of pneumocyte with blood spots and destruction in epithelial layer which lining the inner surface of the alveoli. The tissue section of lung after exposure to 2% concentration of halothane showed lung fibrosis, blood congestion in different locations of lung parenchyma with mild emphysema in wide regions of lung and some sections showed absent the alveolar sac and instead by cystic dilation. The tissue section of lung after exposure to 3% concentration of halothane showed completely degeneration of alveolar wall in different location of lung with proliferation of pneumocyte between the destruction alveoli, high aggregation of inflammatory cells as clusters around the affected alveoli and acute emphysema in some reigns of lung characterized by absent the alveolar sac and instead by very wide cystic dilation.

INTRODUCTION:

Each year in the United States, approximately three million Infants, children and adults receive anesthesia for surgical procedures, with many more receiving anesthesia for imaging studies or dental procedures [1]. The anesthetic agents have been used long ago and it is of great importance the appropriate anesthetic to possess properties like rapid onset, offset of action and predictable metabolism. Among currently used anesthetics are Inhalational anesthetic agents [2]. Inhalational anesthetics are the most commonly used agents for maintenance of general anesthesia in animals and human. The popularity of those drugs to establish anesthesia is based on a range of attractive, such as ease of administration, predictability of their effects, low cost. The most widely used volatile anesthetics cause a dose-dependent depression in respiration and thus have an irritable effect on the airway, and are effective bronchodilators [3]. However, there are also data that general anesthesia could lead to endothelial damage and pulmonary micro vascular injury, which phenomenon could result in respiratory complications after surgery [4]. The anesthetics agents that used at the present time, halothane, enflurane, isoflurane, sevoflurane, desflurane. The appropriate choice of anesthesia for patients, undergoing different type of long-durable surgery intervention requires minimal toxicity and accurate monitoring for patients at high risk for metabolic, cardiovascular and respiratory preoperative complications [2]. The elimination of anesthetic agents should avoid the metabolic breakdown and be independent from renal or hepatic function. Thus they should have minimum undesirable drug interaction, minimum side effects, or toxicity. Although the elimination of volatile anesthetics is primarily through the lung, a certain percent of the inhaled gases is metabolized. Some anesthetics' derivatives have been metabolized in the liver, with production of inorganic fluoride ions. The high tissue inorganic fluoride levels could lead to the nephrotoxic effect [5]. Other volatile halogenated hydrocarbons have been metabolized to

trifluoroacetic acid. The latter interacts with hepatic proteins, induces an immune response in susceptible patients [6]. and lead to hepatotoxic effects. One commonly used inhalation anesthetic, with a known hepatotoxic effect is halothane [7]. Halothane has been used widely as a volatile anesthetic agent in clinical practice since 1957 [8]. It is well established that halothane is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates [9]. Halothane have chemical structures, 2-bromo 2-chloro 1,1,1-trifluoroethane [10]. Its many advantages, such as quick action, easy administration, non-inflammable, high potency and low cost, lead to its widespread use within a short time [11]. About 80% of inhaled halothane is taken by the body, is eliminated unchanged via the lungs, and about 20% of halothane, not eliminated in exhaled gas, is metabolized in the liver to its principal metabolite, trifluoroacetic acid (CF₃COOH) [12]. Halothane is undesirable drug in humans, who risk developing lung disease because epithelial cell apoptosis may stimulate lung fibrosis, acute respiratory distress syndrome (ARDS), acute lung injury, destruction in alveolar wall and emphysema [13]. Halothane may provoke apoptosis in alveolar cells and thus may modulate respiratory function it is of great importance to assess the crucial role of apoptosis in the progression and pathogenesis of lung diseases, which is of value for clinical practice [14].

Aim of Study : 1- Histological changes estimated in lung after exposure to halothane.

MATERIALS AND METHODS:

Experimental Animals:

The present study carried out (80) male albino white mice from drug and healthy center in Baghdad province. The experimental animals were housed in animal house of college of science in Al- Muthanna university under controlling of temperature at 25-28°C with feeding by used standard pellets. The average weight of experimental animals was 30 gm. The experimental animals were divided into four groups. Three treated groups (A,B and C)

and the other (D) as control group. The experimental animals were left in labels separate cages. Each group composed of 20 mice, each group exposure to halothane with or by inhalation for 15 minutes daily during thirty days .

The halothane concentration determined by vaporizer. The oxygen supplies for the vaporizer to mixed with the liquid halothane. The oxygen level for the exposure was 10%

Group (A) : treated with 1 % concentration of halothane .

Group (B) : treated with 2 % concentration of halothane .

Group (C) : treated with 3 % concentration of halothane .



Figure 1: Experimental animals that exposure to halothane anesthesia.

Histological technique:

Fixation: The lung sample was laid in labeled container contain 10% formalin for 48 hours, **Washing:** The sample was washed by using the tap water for (1 hr.), **Dehydration:** The sample was done to remove all extractable water by dehydrated diffusing through the tissue, alcohol was used, **Clearing:** This stage is considered as a middle step, it is obligatory because the used alcohol for dehydration will not dissolve or mix with molten paraffin, **Embedding (Blocking):** Paraffin wax that used is hard (melting point 60-68°C), **Cutting:** cutting was done by using the rotary microtome. The thickness is (5 µm). the sample were put in hot water path with (52°C) for extending tissue, then the samples were carried on the slides which had a thin layer of Mayer egg albumin, the slide was put on the hot plate with (40°C) for overnight, **Staining:** Hematoxylin and Eosin stain: for demonstrating of the general structure of the tissue (15).

RESULTS AND DISCUSSION:

The Histological Result of The Control Group:

The histological results of lung in the control group showed that the tissue sections of lung have normal pneumocyte with dark irregular nuclei centrally location. The tissue section of the lung showed that different generation of bronchial tree have normal wall line by normal epithelial layer. The upper branch of bronchial tree line by respiratory epithelia simple columnar epithelia. The present result of lung showed high amount of elastic fibers which distributed

between the different generation of bronchial tree (figure 2). The current results of lung showed the upper generation of bronchial tree lined by different types of epithelia started with simple cuboidal epithelia, while in lower generation of bronchial tree lined by simple squamous epithelia. The alveolar sac have thin wall and line by simple squamous epithelia. The alveolar sac were surrounded by network of blood capillaries (figure 2).

The Histological Results of Lung After Exposure to 1% Concentration of Halothane :

The tissue section of lung that exposure to 1% concentration of halothane have some alveoli destruction and aggregation of inflammatory cells around the cystic dilation, so showed small aggregation of pulmonary alveolar macrophage (PAM) in some of cystic dilation (figure 3). This result occur because toxicity of halothane which causes this changes ,this findings agreement with [16] which said that The histological changes of halothane showed increase region of destruction, inflation and dilation of airway. The histological sections of lung showed that destruction in epithelial layer which lining the inner surface of the alveoli. The present result of the lung showed that small clusters of inflammatory cells between the alveoli. The present results of lung showed prominent emphysema, with proliferation of pneumocyte with blood spots. The alveolar sac characterized by disappeared the epithelial cell that lining the internal surface of alveolar sac (figure 3). The results showed bronchiole filled with secretion and blood (figure 3). This results noted that the halothane affected on the epithelial layer of bronchial tree and alveolar sac. The present results were similar to [17,18] which said that anesthesia have effects on the respiratory system, general anesthesia causes decrease in respiratory rate and respiratory complications by mismatch in alveolar breathing leading to the destruction of the alveolar wall and thus the occurrence of pulmonary complications after surgical operations.

The Histological Results of Lung After Exposure to 2 % Concentration of Halothane :

The histological results of lung section after treated with halothane 2% concentration showed blood congestion in different locations of lung parenchyma with mild emphysema in some reigns of lung characterized by absent the alveolar sac and instead by cystic dilation (figure 4). The high concentration of halothane which lead to acute histological destruction in the alveolar sac. This result were agreement with [14] which said that halothane may provoke apoptosis in alveolar cells and thus may modulate respiratory function it is of great importance to assess the crucial role of apoptosis in the progression and pathogenesis of lung diseases, which is of value for clinical practice. The most histological sections of the lung showed the alveoli and terminal bronchial tree have aggregation of RBCs (figure 4). The present result of lung showed separated the epithelial layer from basal line of bronchial tree, and some epithelial cells exfoliated and aggregation in the lumen of bronchial tree (figure 4). The present result showed blood hemorrhage in different location of the lung (figure 4). The results showed lung fibrosis in different regions of lung after treated with 2% concentration of

halothane (figure 4). This result similar to [13] which said that halothane is undesirable drug in humans, who risk developing lung disease because epithelial cell apoptosis may stimulate lung fibrosis, acute respiratory distress

syndrome (ARDS), acute lung injury ,destruction in alveolar wall and emphysema.

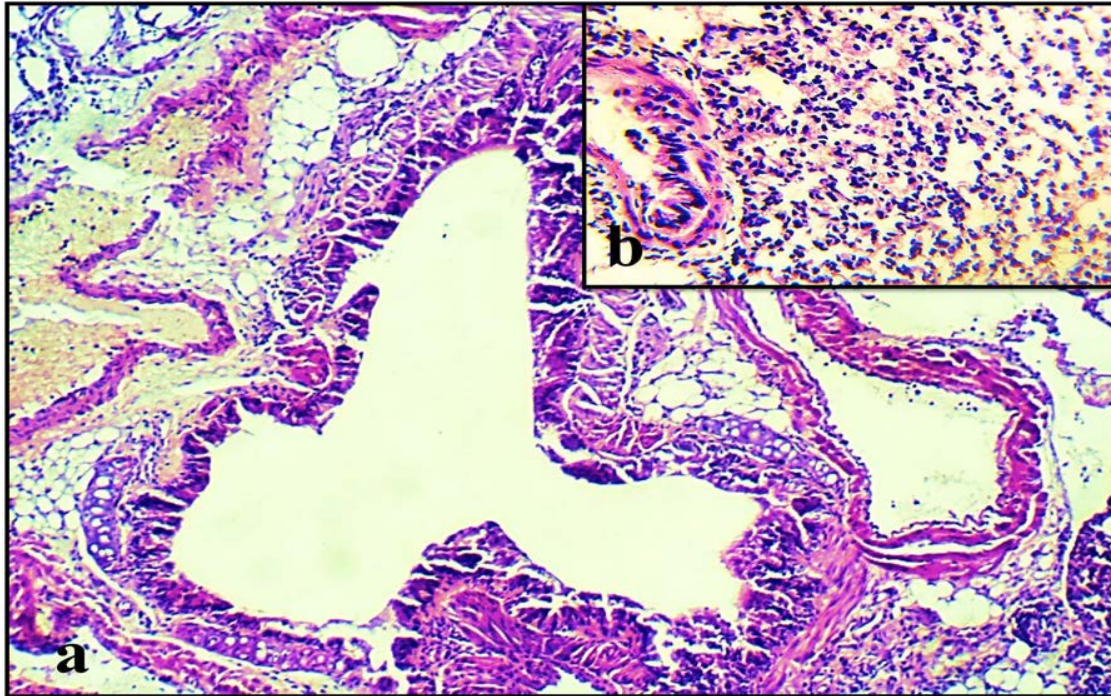


Figure 2: cross section of lung in control group. **H&E** stain **a**: 10X , **b**: 40X.

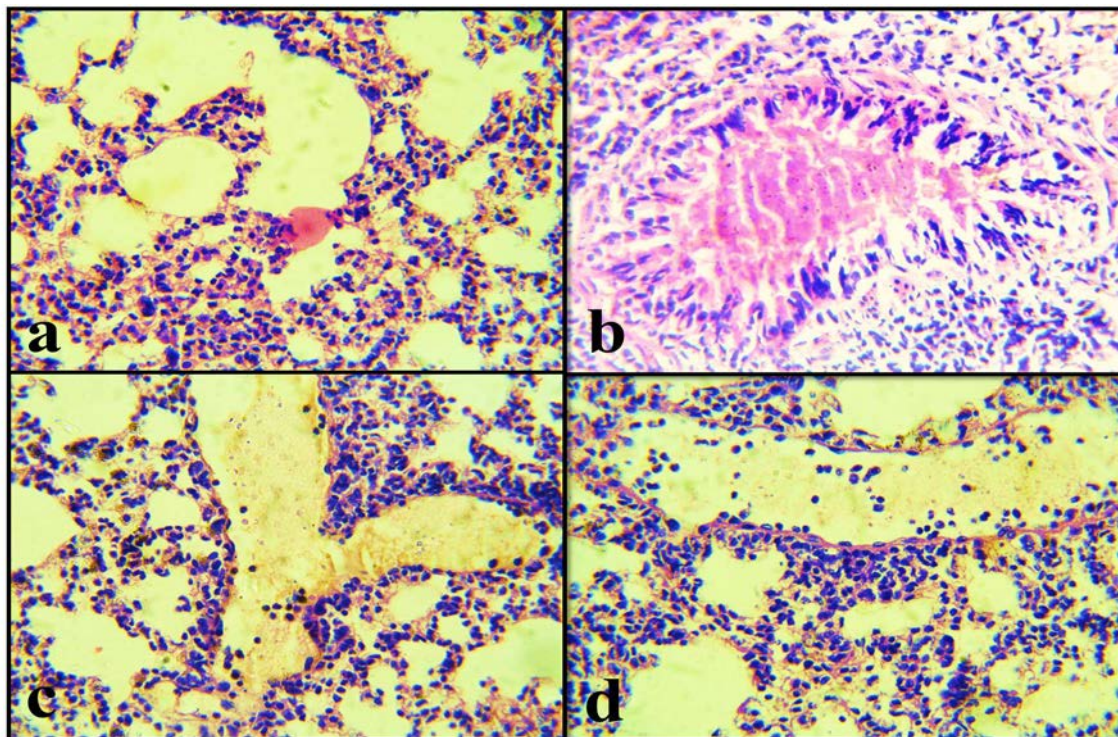


Figure 3: cross section of lung after exposure to 1% concentration of halothane. **H&E** stain **a**, **b**, **c**, **d**: 40X .

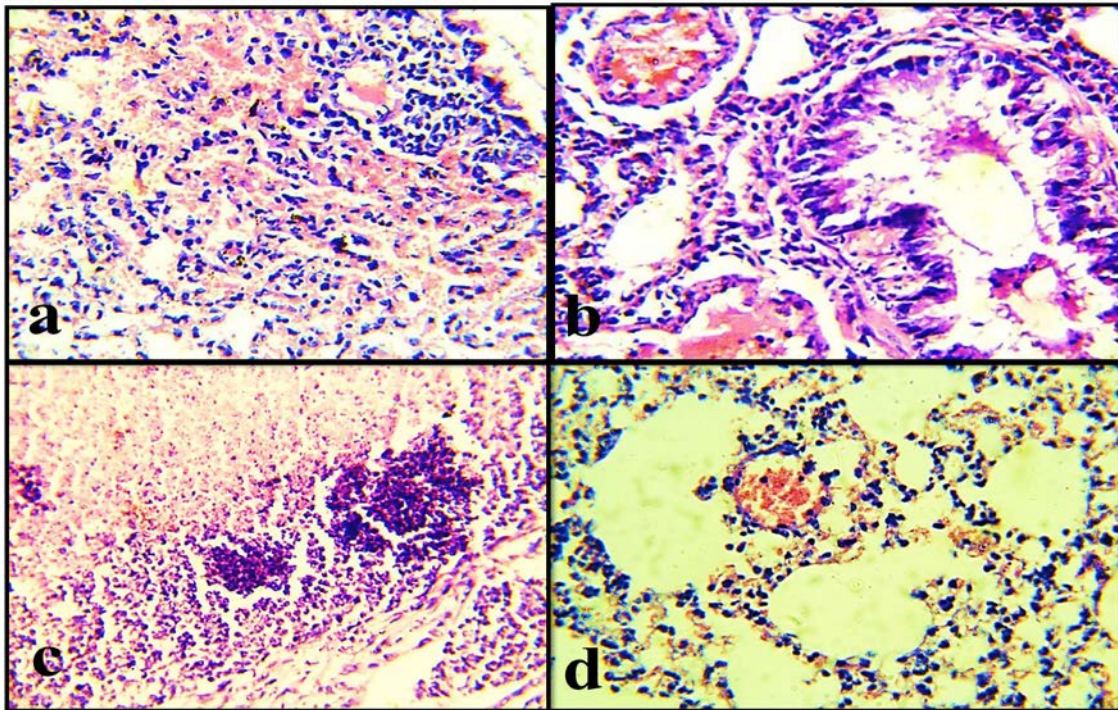


Figure 4: cross section of lung after exposure to 2% concentration of halothane. H&E stain a, b, c, d: 40X .

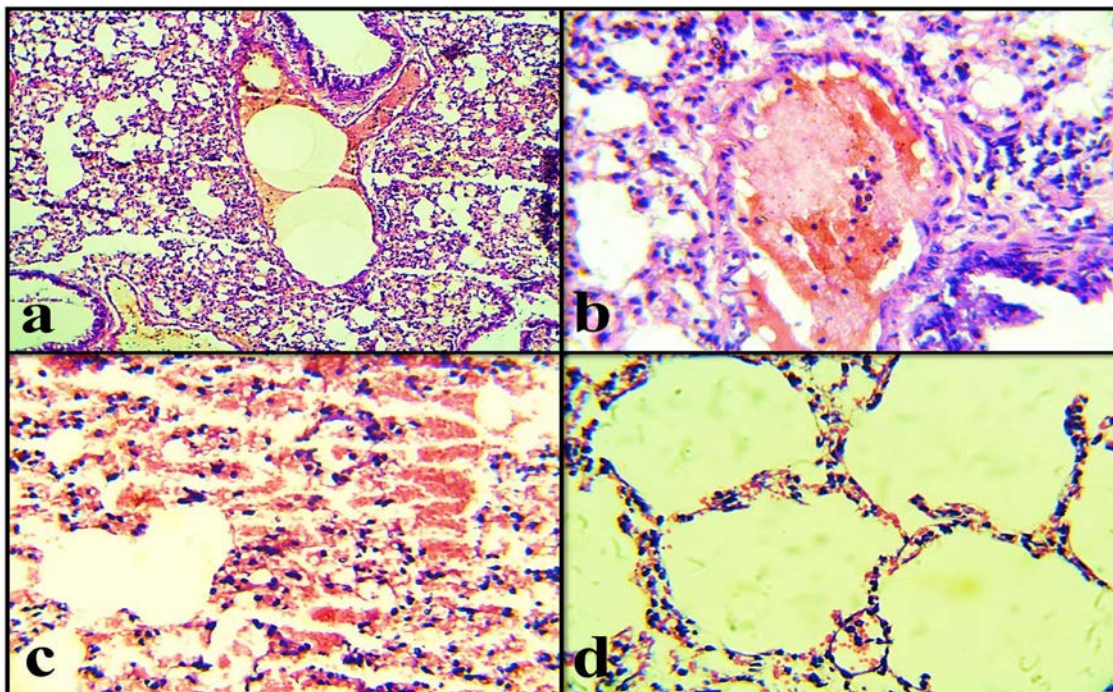


Figure 5: cross section of lung after exposure to 3% concentration of halothane. H&E stain a, b, c, d: 40X .

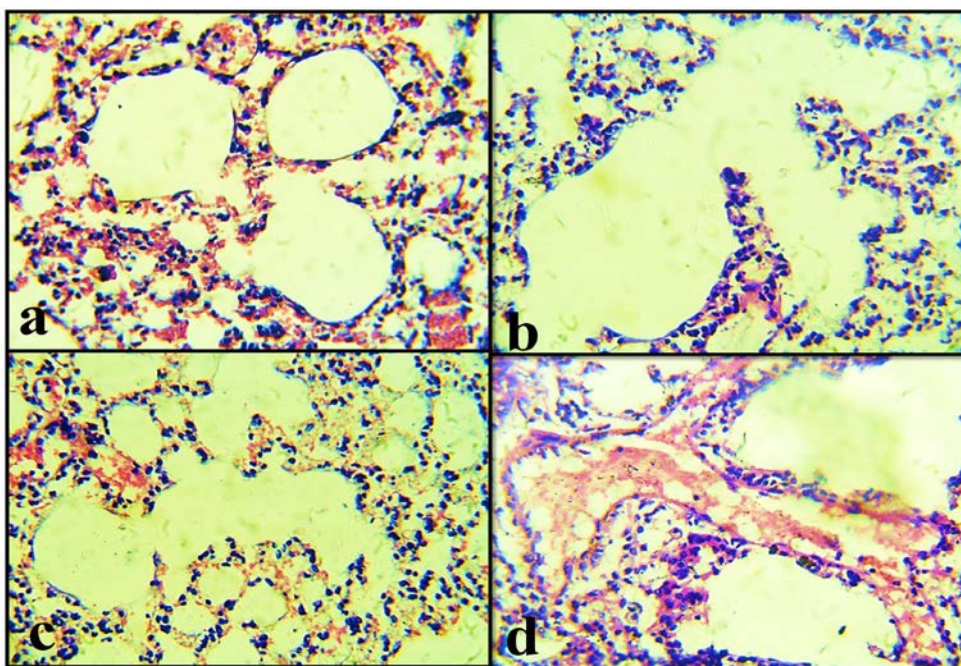


Figure 6: cross section of lung after exposure to 3% concentration of halothane. H&E stain a, b, c, d: 40X .

The Histological Results of Lung After Exposure to 3 % Concentration of Halothane :

The lung of third groups which inhaled the halothane 3 % concentration showed completely degeneration of alveolar wall in different location of lung with blood congestion between the destruction alveoli (figure 5,6). The cross section of lung tissue after exposure to halothane, showed high aggregation of inflammatory cells as clusters around the effected alveoli. The current result of lung after treated showed the conduction zone of bronchial tree filled with thick secretion and have large aggregation of pulmonary alveolar macrophage inside the lumen of this zone (figure 5,6). This result agreement with [19] that said general anesthesia have side effects on the airway, this effect including relaxation of muscles and loss of cough reflex with full the airway with secretions causes in airway prevention, and dry gases effect of the function of respiratory system. The histological result of lung section after treated with halothane 3% concentration showed blood hemorrhage in different locations of lung parenchyma with acute emphysema in some reign of lung characterized by absent the alveolar sac and instead by cystic dilation (figure 5,6). This result agreement with [13] which said that halothane is undesirable drug in humans, who risk developing lung disease because epithelial cell apoptosis may stimulate lung fibrosis, acute respiratory distress syndrome (ARDS), acute lung injury, destruction in alveolar wall and emphysema.

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