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# Study of the effect of pesticide 2,4-D on the histological structure of the lungs in the albino mice (*Mus musculus*) .

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**Abstract**

**Objective**: Study of the effect of pesticide 2,4-D on the histological structure of the lungs in the albino mice (*Mus musculus*)

**Methods**: The study involved the use of (20) male mice. The animals were divided into three groups, the first one was control group included the mice that dosage with physiological saline solution, while the other group included the mice that treated with 150 mg/kg 2,4-D. the final group was consist of mice treated with 200 mg/kg of 2,4-D. then the animals were dissected after 30 days for the removal of lungs, then it was fixed by fixative solutions after that a serial of histological sections preparations were conducted.

**Result**: Structural changes were found in the experimental pesticide 2,4-D group compared to the controls, showed that the lung has been necrosis of alveolar and alveolar sacs cells as well as an enlarged cavity and the appearance of spaces between the mucosa and submucosa tunica in the respiratory bronchioles, And also fibrosis in the surrounding smooth muscle fibers . It was also observed that there was inflammatory cells, edema, and space appear between the mucosa and submucosa layer of the bronchioles terminated.

**Conclusion**: pesticide 2,4-D has toxic effects on the lung tissue resulting in interstitial fibrosis.

**Keywords:** pesticide, alveolar, Lung, pulmonary blood vessels, Interstitial fibrosis.

## INTRODUCTION:

The use of Herbs was widely used in all over the world on the last sixty years ago [1]. And in recent years. Concerns were raised about the apparent effects and potential adverse effects of various environmental pollutants, particularly pesticides, on human, animal and environmental health as a result of their residues of chemicals classified as residues of xenobiotics (a substance that is not produced but is present in the body of the organism) After exposure to any chemical. One of these effects of Herbs that were clearly on different human's systems and organs, the great effect on the structure and function of the human Respiratory system especially lungs[2].

Generally, the effect of herbicides is low to moderate toxicity in the human and animal body because most of them attacked the chemical pathways of the plants, However, the residue of pesticides accumulated in various elements of the environment as a whole. However, their residue accumulated in various elements of the environment like air, water, soil, and food or when exposed to a certain Concentrations from it. Their side effects increased which appeared in the different living organism when predisposing factors exist, like inhalation of a spray of volatile pesticides because of a cough and Burning in nasal passages.and chest. Coma occurred when inhalation process prolong for longer time and caused vomiting , Heartburn , diarrhea and muscle tremor when it swallowed

Represents the use of Herbicides 36%, insecticides 25% and Fungicides

10% Of the global use of different pesticides.[3,4]. Over the past decades, many herbicides have been widely used and are currently being used to control weeds But without taking into account their serious toxic effects on both living organisms and the environment [4]. 2,4-D 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide, considered one of the most successful herbicides used in the world and the most widely used in modern agriculture. It is a chemical compound that contains two main constituent parts: phenol (phenoxyphenoxy) the element of chlorine (dichloro-) and both parts give this pesticide its acidic effect. Previously, a 2,4-D pesticide was used with high concentrations to control many broad-leafed grass species in grasslands, gardens, agricultural fields and forests [5]. The toxic effects of pesticide 2,4-D have been documented in many scientific studies, where exposure to pesticide 2,4-D has been shown to result in a variety of damage to rodents, such as genetic damage [6], hepatic [7] and neuralgia [8] and renal toxicity [9]. Also, the respiratory system is very sensitive to many chemicals in the environment that contribute in one way or another to cause tumors in the respiratory system [10]. It was found that herbicide 2, 4-D increases fat oxidation in both animal and human cells [12, 11]. It also causes cellular mutations that can lead to certain types of cancer. This pesticide (which behaves in the behavior of the mutagen) contains dioxin compounds and a group of known chemicals that are dangerous to human health and the environment. In addition to all the previous effects, there was a clear increase and significant increase in birth defect rates among neonates born to the population of the agricultural areas where pesticide 2 is used , 4-D and other herbicides of the same class [15].

However, few attempts have been made to monitor the effect of a 2.4-D pesticide on the respiratory system that can function either directly or indirectly on this sensitive organ of the human body. [16]

The aim of the current study is to determine the effect of pesticide 2-4-D on the tissue structure of the lungs in the *(Mus musculus* ) white mouse, which gives a clear impression and a very close picture of the effect of this pesticide on humans due to the high similarity in the genetic map between humans and mice . The aim of this study was to evaluate the effect of 2,4-Dichlorophenoxyacetic acid pesticide (abbreviated as 2,4-D) on the tissue composition of the lungs in Swiss MICE *(Mus musculus* ) .

## METHODOLOGY

The pesticide dose used was prepared depending on half lethal dose ($LD_{50}$) which is 370 mg/kg in mice according to(16) (**Kaulbars and Vaillancourt, 2014**). Concentration of 150 mg/kg was selected and the weights of mice were ranged between 20-30 g. The treated doses were prepared according to the following relation:

$$\frac{1000}{W_m} = \frac{D}{x}$$

Where: $D$: The selected dose in (mg/kg), $x$: The dose according to mouse weight and $W_m$ : Weight of mouse in (g).

The mice were treated with the used dosage of 2,4-D pesticide once daily for one month for each treated concentration.

**Laboratory Animals and Histological Study**:

Twenty male mice of Swiss mice (*Mus musculus*) were obtained from animal house in Biology Department-College of Education

for Pure Science/Diyala University. The average weights and ages were between 20-30 g and 8-10 weeks respectively. The experiments animals were divided randomly into three groups as follow: 1st group (CG) is control group and has 8 male mice and 2nd group (EG) is experimental group and has 16 male mice. The second group is sub divided into two groups of 8 male mice for each one. The animals in one of these sub-group (EG1) were treated with 150 mg/kg of 2,4-D pesticide while the mice in the other sub-group (EG2) were treated with 200 mg /kg of 2,4-D pesticide. The animals were treated once daily for 30 days. In the end of the last day of dosage, all mice were anesthetized with chloroform, post-mortem to eradication of kidney, fixed with formalin for 24 h, washed with tap water and transferred to 70% alcoholic solution for keeping. Histological sections were prepared depending on (18) (**Bancroft and Gamble, 2008**). The sections were (Dehydration) with an ascending chain of ethyl alcohol, then immersed in xylene for (clearing) and embedding in paraffin wax. The wax molds were cut using rotary microtome with 7µm thickness. The sections was stain used hematoxylin and eosin stain according to (19) (Suvarna *et al*., 2013) and finally loaded with Canada balsam, tested and photographed using light microscope supplied by digital camera.

## RESULTS AND DISCUSSION

The current study shows that the lungs tissue's in the control group of mice are mainly composed of the alveoli and alveolar sacs, which give the lungs a spongy appearance. Figure (1) shows the final part of the respiratory tree, which opens a number of alveoli, alveolar and alveolar channels, which are lined with a simple columnar ciliated epithelium tissue without goblet cells. while alveolar and alveolar sacs are lined with Simple Squamous epithelial tissue and a small portion of cubic or round cells. A small combination of smooth muscles and elastic fibers around the bronchial pore and alveolar channels was also observed, this result is agreed with a study by researchers (20).

The results of the histological study which conducted on the mice treated with 150 mg/kg of D-2,4 showed that the lung has been necrosis of alveolar and alveolar sacs cells as well as an enlarged cavity and the appearance of spaces between the mucosa and submucosa tunica in the respiratory bronchioles, And also fibrosis in the surrounding smooth muscle fibers, as shown in Figures (2,3)

These changes associated with some of the cells of the walls of alveolar can be said to have come from the effect of toxic chemical compounds established in the pesticide [6]. The researcher (21) noted that cell necrosis may be caused either by progressive degeneration of the enzymes within the infected cells, or metabolic disturbance, or inhibiting the synthesis of DNA and thereby inhibiting the synthesis of the proteins necessary for cell growth and maturation. While the researchers (22) indicated that such tissue changes can be attributed to the reduction of antioxidants in the animal's body due to the chemical.

It was also observed from the obtained results of this study that a blockage made in the blood vessels because of blood clot and rupture in the wall of blood vessels. It was also observed that gaps in the vesicles of cellular transport inside the cavity of the vessel indicating the effectiveness of high vascular lining as in the Figures (4).

The congestion in the pulmonary blood vessels may have been caused by excess blood in the venous system, leading to increased blood pressure in the veins and capillaries. This may exert undue pressure on the surrounding structures, usually accompanied by low blood supply, Thus, the cells are exposed to malnutrition, lack of oxygen and accumulation of waste materials (23).

The results are shown in Figure (4) indicate that inflammatory cells, edema, and space appear between the mucosa and submucosa layer of the bronchioles terminated. The presence of chemical substances and the development of reactions lead to increased vascularization of low-protein fluids and thus accumulate in the intercellular distances and produce edema (24)

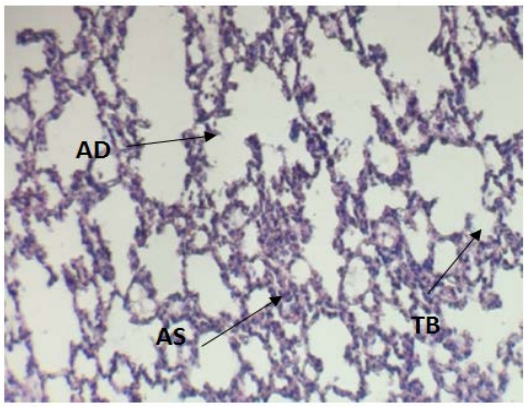


**Figure ( 1) )** Parasagittal section of mice lung of the control group showing the AD alveolar duct AS alveolar spaces and TB terminal bronchiole H&E x 400).

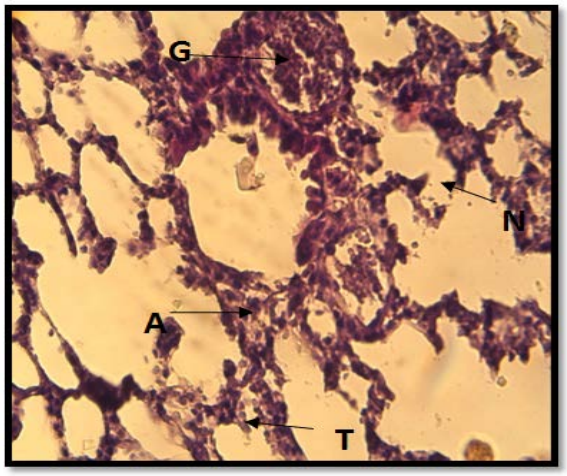


**Figure ( 2)** Parasagittal section of the lung of mice receiving 2,4-D 150 mg/kg for one month showing A, inflammatory cells. G, coagulate .T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs H&E x 400).

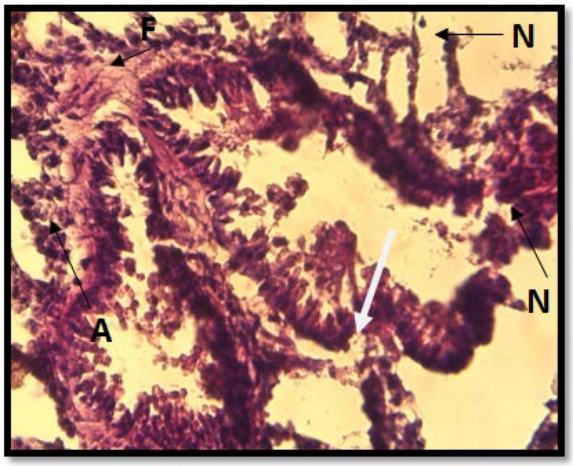


**Figure ( 3)** Parasagittal section of the lung of mice receiving 2,4-D 150 mg/kg for one month showing A, inflammatory cells. F, fibrosis in the surrounding smooth muscle fibers.T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs H&E x 400).

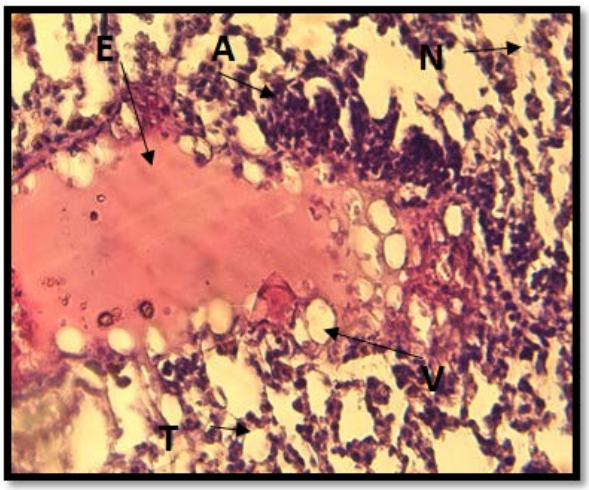


**Figure ( 4)** Parasagittal section of the lung of mice receiving 2,4-D 150 mg/kg for one month showing A, inflammatory cells. V, Vesicles of effective transport.T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs .E, Edema .H&E x 400).

The side effects of the pesticide are increased by increasing the concentration of the pesticide. The mice that were treated with D-2,4 at a concentration of 200 mg/kg showed more negative effects than the previous concentration. Where it was characterized by greater expansion in the cavity of alveolar and alveolar sacs and necrosis of cells, and the occurrence of rupture in some walls and in the walls of capillary blood vessels as in Figure (5).

Chemical compounds have an important activate in the deform and coagulation of proteins. These compounds may have superficial recipients on the surfaces of the cells that make up the walls of the alveoli and alveoli sacs, which have affected the cell proteins, causing them to necrosis and degenerate. Necrotic tissue is a foreign body and acts as an irritant, irritating agent and triggers inflammatory reactions in adjacent tissues. That dead bronchial cells release hydrolysis enzymes on large complex molecules and turn them into smaller molecules, followed by the effect of inflammatory cells in capturing these molecules followed by the effect of inflammatory cells in capturing these molecules. This may be one of the causes of rupture in the walls of alveolar and alveolar sacs (25).

The histology sections of the lung showed an increase in the thickness of the walls alveoli in other regions, which led to closing the cavity. Also it was observed a bleeding and blood clots were . Also,observed, and gaps were observed in the vesicles of the cell transport inside the vessel cavity indicating high activity of the lining of blood vessels as described in Figure (6).

The increase in the thickness of the walls of the alveolar in some areas and the reduction of cavity, which may have been the result of the expansion of the alveoli and alveolar sacs, which has caused the expansion of pressure on the constituents of the lining of the lung, which increased the compression and make them characterized by increasing the thickness of the walls and the decrease of cavity (26).

Other manifestations of a blood clot that closes some capillaries in the lungs of rats treated with the pesticide are due to the effect of chemicals in the deposition of proteins, which may cause the deposition of blood proteins.

A distinctive feature in the tissue sections of lungs of mice treated with the pesticide at concentration of 200 mg/kg is the increased efficacy of endothelial lining of the blood vessels in the transfer of inflammatory cells and this appears in vaculation form. This explains the presence of inflammatory cells whose presence indicates their important effect in defending the body and attempting cellular repair. (26, 28)

The current study also showed the infiltration of inflammatory cells within the cells, and the emergence of collagen fibers more than in previous transactions, which indicate that there is fibrosis and the emergence of a space between the mucosa and submucosa layer of the bronchioles terminated as in Figure (7

As the inflammation continues, these cells stimulate the alveolar cells to divide into fibroblasts by catalysts. These fibroblasts begin to produce collagen fibers that accumulate without differentiation. Thus, pulmonary fibrosis, which occurs in some affected tissue areas in mice treated with pesticide concentrations (26)

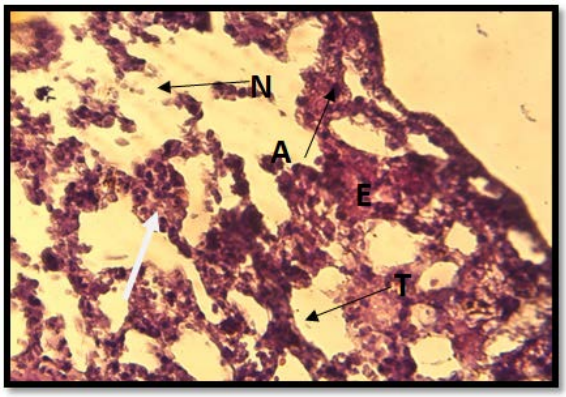


**Figure (5)** Parasagittal section of the lung of mice receiving 2,4-D 200 mg/kg for one month showing A, inflammatory cells..T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs .E, Edema .H&E x 400).

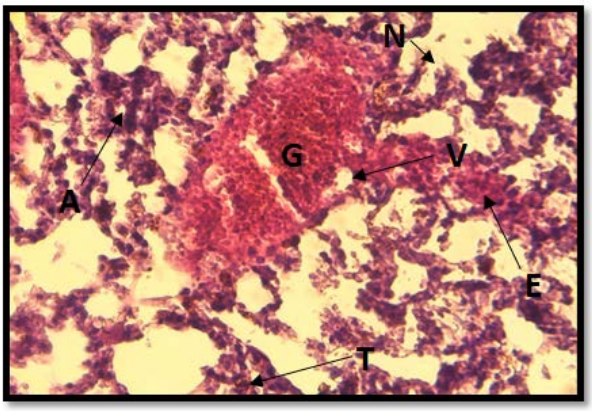


**Figure ( 6)** Parasagittal section of the lung of mice receiving 2,4-D 200 mg/kg for one month showing A, inflammatory cells. V, Vesicles of effective transport .T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs. E, Edema. G, Congestion .H&E x 400).

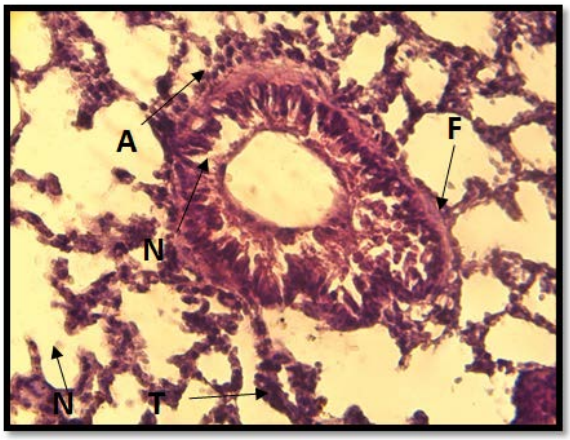


**Figure ( 7)** Parasagittal section of the lung of mice receiving 2,4-D 200 mg/kg for one month showing A, inflammatory cells. F, fibrosis in the surrounding smooth muscle fibers.T, thickness of the walls alveoli N necrosis of alveolar and alveolar sacs H&E x 400).

## CONCLUSION:

Pesticide 2,4-D has toxic effects on the lung tissue resulting in interstitial fibrosis and necrosis of alveolar and alveolar sacs cells.

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