

## **THE HISTOPATHOLOGICAL CHANGES OF INHALATION OF TOLUENE IN RABBITS**

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**Keywords;** rabbits, alveolar wall, oedema.

### **ABSTRACT**

This study was designed to determine whether toluene abuse affected the organs function or general health of males and females. In this study used 24 rabbits of both sex , the animals were divided in four groups , six animals in each group ,the animals were exposed to toluene vapor inhalation ( 500 ppm / 2h/day ) daily for 2 months.

Exposure –related suppression of body weight gain and food consumption were observed. Salivation and lacrimation were observed during exposure periods .

The pathological changes these were showed organs have simple different between the males and females. The pathological changes observed in the lungs of males are emphysema, infiltration of lymphocytes in alveolar wall , thickness of alveolar wall ,and hemorrhage ,similar to that in female .

The pathological changes observed in the kidney of females observed fatty degeneration and cloudy swelling ,while in the males cloudy swelling only. The pathological changes observed in the liver of females observed hydropic degeneration , while in the liver of males hydropic degeneration , congestion of blood vessels ,and infiltration of inflammatory cells (lymphocytes).

The pathological changes observed in the uterus of females observed fibrosis, oedema , degeneration of the cells lining the uterine gland ,and red blood cells with inflammatory cells.

### **INTRODUCTION**

Toluene is a widely abused inhaled solvent . Toluene is the chemical name of formula  $C_6H_5CH_3$ , the synonyms methylbenzene, phenylmethane ,methacide , methylbenzol .Toluene belongs to a large class of chemicals called organic solvents. Alcohols, acetone, methyl ethyl ketone, trichloroethane, and xylene are a few other

examples of organic solvents. Toluene is sometimes called "toluol .It is also used explosives ,dyes and other organic compounds as solvent for paint ,gums ,most [1] .

A wide variety of chemicals can be abused as inhalants. The products used for common household and industrial purposes. These include cigarette light gas ,cleaning fluid, spray paint ,paint thinners and removers,correction fluid ,nail polish remover,pet, glues , gasoline [2]. Inhalants are readily absorbed into the lung and produce an altered mental state within seconds ,but the effects last for only about 5 to 15 minutes [3].

The exact mechanisms of action for volatiles are unknown ,but common theories are generalized slowing of axonal ion channel transport and potentiation of hyperpolarization of gamma-amino butyric acid receptors [4]. Chronic inhalant abuse can damage cardiac, renal ,hepatic ,and neurologic system. Inhalant abuse may result in bone marrow suppression, leading to leucopenia ,anemia ,thrombocytopenia ,and hemolysis [5]. Chronic inhalation of toluene may also associated with substantial hematological , renal, hepatic ,and neurologic morbidity and mortality [ 6,7,8 ]. Several studies in both non-pregnant humans and animals have demonstrated that toluene absorbed into the blood is distributed throughout the body with the brain ,liver ,containing the highest level [9,10,11].

Aims of the present study is to assess toxic effects of toluene inhalation to the organ in rabbits.

## **MATERIAL AND METHODS**

**Chemical :-** The toluene used in this experiment was obtained from the Chemistry Department , College of Science ,University of Basrah .

**Animals :-** The animals used in this study were 24 local mature males and females rabbits(12 rabbits for one sex ). Weighting ( 1.5 - 2 Kg) and aging relatively between (7-10 ) months .The animals divided into four equal groups randomly, six animals in each group .First group regarded as female control group (G1) . Second group regarded as male control group (G2) . Third males group (G3)was exposed to toluene vapor inhalation (500 ppm / 2h / day )daily for 60 days . Fourth females group (G4) was exposed to toluene vapor inhalation for (500 ppm / 2h / day )daily for 60 days [ 12 ].

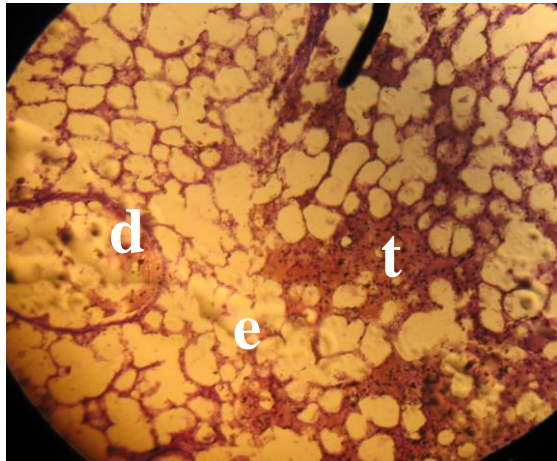
Animals were maintained in air conditioned quarter (24 °C) under stander husbandry condition with alterte 12 h. light / dark period , and were given prepared ration composed of wheat flour, bran ,mash powdered milk ,and 1% NaCl in addition to green alfalfa and tab water *ad libium* .The following criteria were studied :-

**1.Histopathological samples :-** the samples obtained from the following organs ,lungs ,kidneys ,livers ,and uteruses .These tissues fixed with formalin then dehydrated by graded alcohol ,this is followed by dealcoholization with xylol and impeding with paraffin wax and blocking . Histological sections of 5-6  $\mu$  thickness were obtained by a microtom ,dewaxed , dehydration and stained by Hematoxylin and Eosin stain , from all animals of all groups [13].

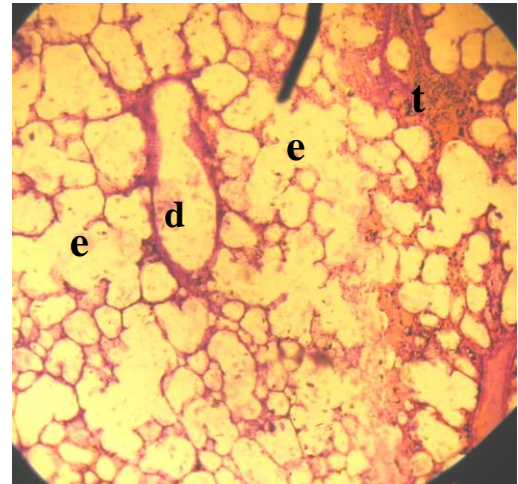
**2.The effect on body weight :-** the rabbits in each groups were weighted at beginning of the experiment then every weeks and continue to the end of the experiment which lasted for 8 weeks.

## **RESULT**

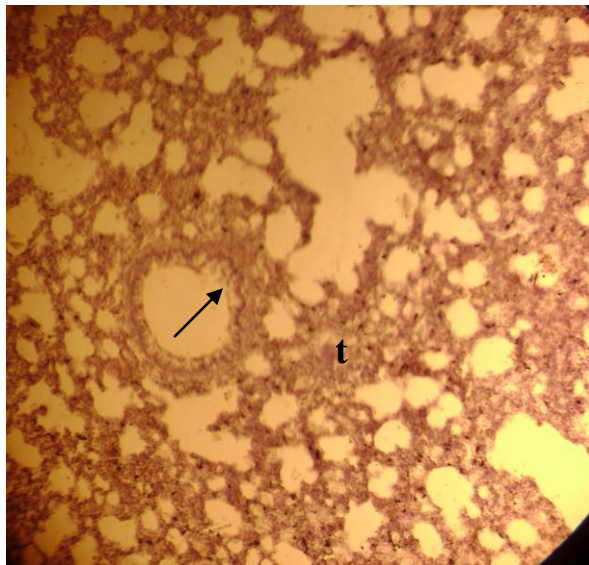
The histopathological changes in the different organs were revealed that the females more effected by the toluene than the males .The changes observed in the lungs of treated males (G3), there is dilatation of bronchioles , areas of interstitial pneumonia , emphysema (distraction of aliveolar walls in some areas that composed large spaces ) , infiltration of lymphocytes into aliveolar wall and hemorrhage lead to thickening in the wall [Figer 1].While the lungs of treated females (G4) shows , emphysema , fibrin into the aliveolies and hemorrhage , thickening into the wall , evidence of proliferation of bronchial epithelium associated with folding of the epithelium lumen ( papillary – like projection ) ,and proliferation of inflammatory cells [Figer 2 ,3 ] compared with control group [Figer 4 ] .



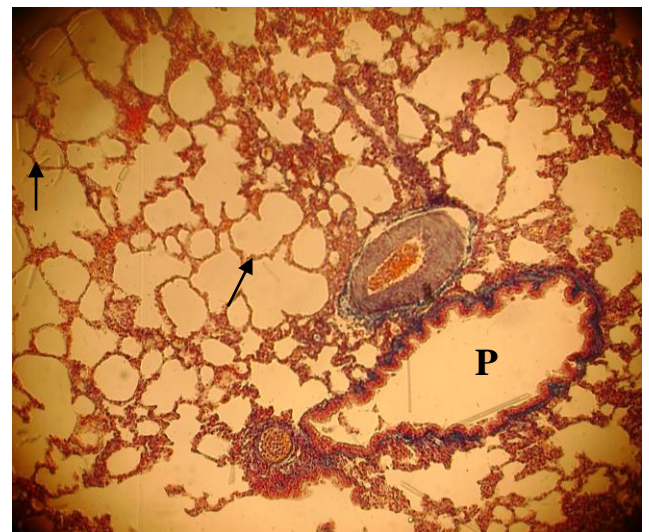
**Figur 1:** section of treated male lung shows ,(d) dilatation of bronchioles ,(e) emphysema , (t) thickening of alveolar walls , 100X .



**Figur 2:** section of treated female lung shows ,(d) dilatation of bronchioles ,(e) emphysema , (t) thickening of alveolar walls , 100X .



**Figur 3:** section of treated female lung shows , (t) thickening of alveolar walls ,proliferation of bronchial epithelium (arrow) 100X .



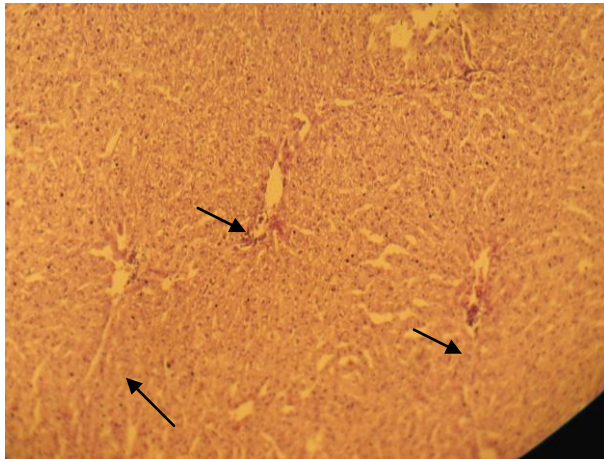
**Figur 4 :** section of control lung shows, thin alveolar walls (arrows) , bronchi ( P ) 100X .

The changes observed in the livers of ( G3) shows ,hydropic degeneration ( ballooning cells or larged cells due to vacuolated cytoplasms with presence of nucleus in the center of cell) , congestion of blood vessels , and infiltration of inflammatory cells (lymphocytes) [ Figur 6]. While the livers of ( G4) shows , hydropic degeneration ,and slight periportal fibrosis[ Figur 7 ] compared with control group [Figur 5 ] .

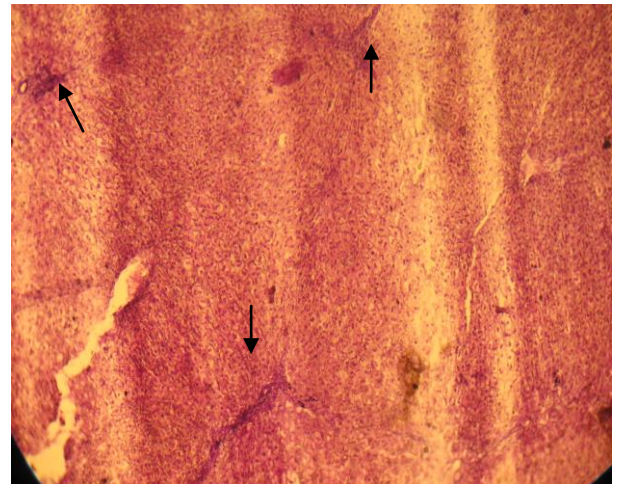
The changes observed in the kidneys of ( G3 ) shows ,cloudy swelling of some renal tubules cells that mean (the cells of this tubules enlarged and become circulatory with



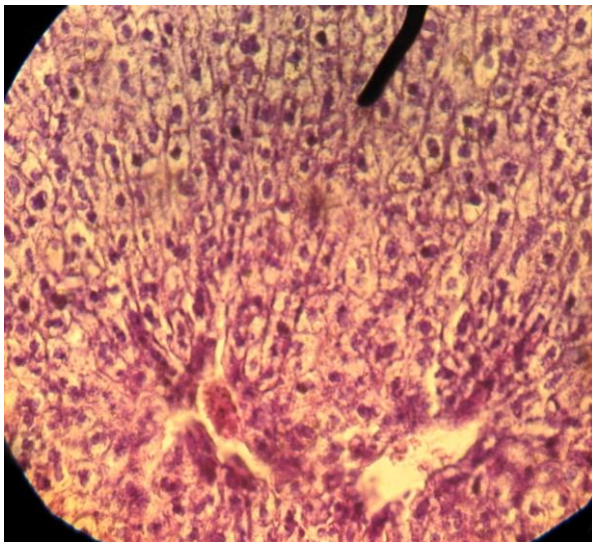
narrowing lumen of renal tubules) [ Figur 9]. While the kidneys of ( G4 ) shows, cloudy swelling of some renal tubules cells ,and fatty degeneration that mean the cells of this tubules enlarged with presence of nucleus in the peripherals because of the fatty drops occupied the cytoplasm that push the nucleus to peripherals [ Figur 10 ] compared with control group [Figur 8 ].



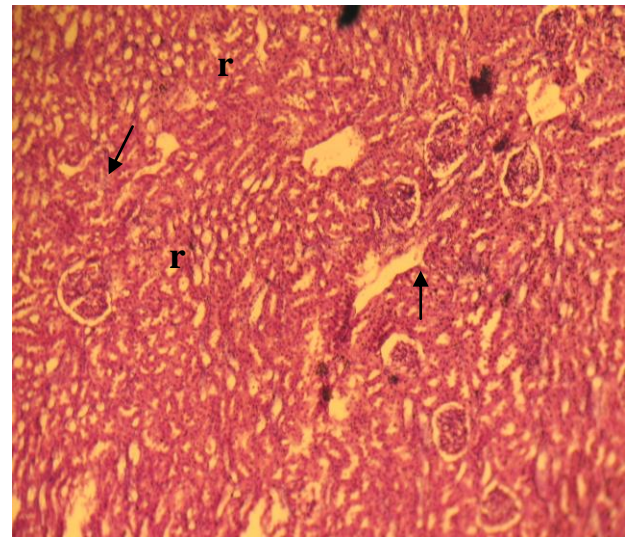
**Figur 5:** section of control liver shows , hepatic cells , and central veins (arrows ) , 100X .



**Figur 6:** section of treated male liver shows , infiltration of inflammatory cells (arrows) , and hydropic hepatic cells , 100X .

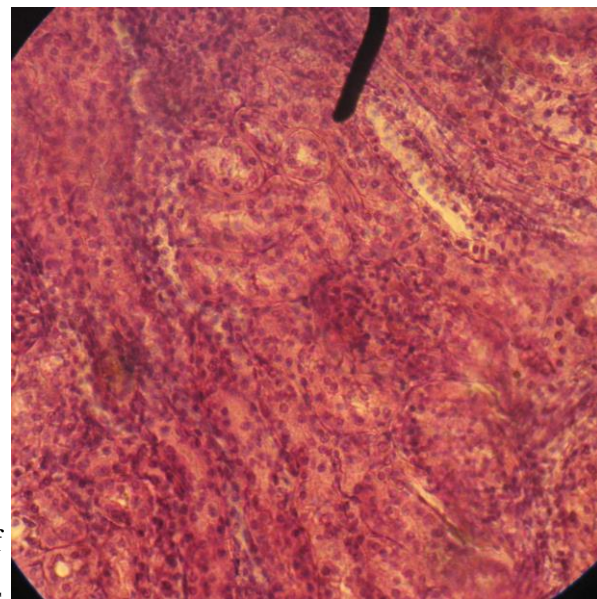
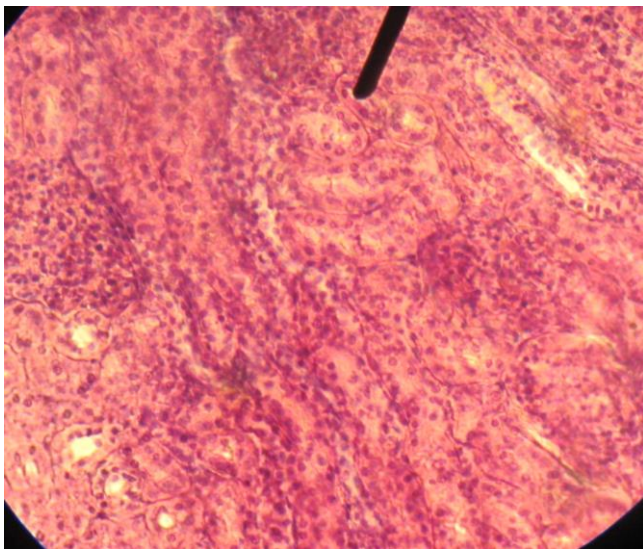


**Figur 7 :** section of treated female liver shows , hydropic hepatic cells (pointer) , congested blood vessels (arrow) , 400X .



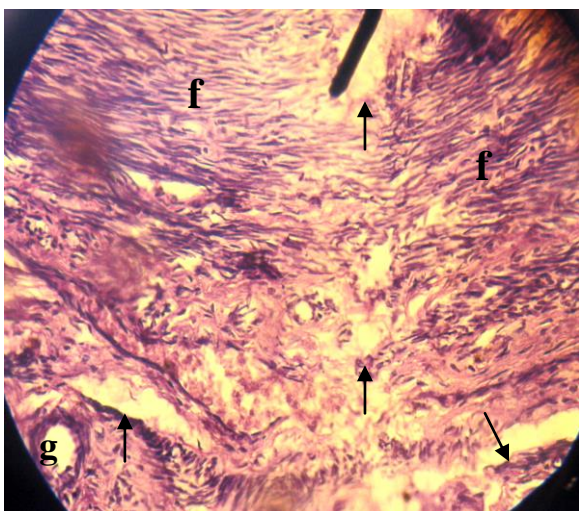
**Figur 8 :** section of control kidney shows, renal tubules ( r ) , glomerulus's ( arrows ) , 100X .





**Figure 9:** section of treated female kidney compared with control group [Figure 12.].  
**Figure 10:** section of treated female kidney shows swelling renal tubules (pointer), 400X.

**Figure 10:** section of treated female kidney shows swelling renal tubules (pointer), 400X.



**Figure 11:** section of treated female uterus shows, oedema ( arrows), fibrosis (f ), uterine gland ( g ), 400X.

**Figure 12:** section of control female uterus shows, uterine gland ( arrows), muscular layer (M) and endometrial layer ( e), 100X.

A significant decrease in the body weight of treated females group with toluene ( $p < 0.01$ ) [ Table 1] compared with female control group in the fifth and seventh weeks .The result obtained into [ Table1 ] shows the decrease in the body weight of treated males with toluene (500 ppm ) compared with control group in the seventh and eight weeks .

**Table 1 : Effect inhalation of toluene on the body weight of treated females and males rabbits with toluene (500 ppm)**

(N=6) , ( Mean  $\pm$  SD ).

week	0week	1week	2week	3week	4week	5week	6week	7week	8week
<b>Male</b>	Aa 1.5583 $\pm 0.3761$	Aa 1.5350 $\pm 0.3675$	Aa 1.4950 $\pm 0.3720$	Aa 1.4517 $\pm 0.3615$	Aa 1.4100 $\pm 0.3558$	Aa 1.3733 $\pm 0.3492$	Aa 1.4217 $\pm 0.3400$	Aa 1.3067* $\pm 0.3180$	Aa 1.2367* $\pm 0.3358$
<b>Male control</b>	Aa 1.6250 $\pm 0.4034$	Aa 1.7317 $\pm 0.2735$	Aa 1.8567 $\pm 0.3146$	Aa 1.8650 $\pm 0.5719$	Aa 1.8667 $\pm 0.5516$	Aa 1.8033 $\pm 0.3986$	Aa 1.8817 $\pm 0.4408$	Ab 1.9383 $\pm 0.4716$	Ab 1.9717 $\pm 0.5501$
<b>female</b>	Aa 1.5833 $\pm 0.3235$	Aa 1.5117 $\pm 0.2901$	Aa 1.4667 $\pm 0.2878$	Aa 1.3983 $\pm 0.2739$	Aa 1.485 $\pm 0.2895$	Aa 1.4000* $\pm 0.2098$	Aa 1.7083 $\pm 0.2840$	Aa 1.2000* $\pm 0.3062$	Aa 1.2067 $\pm 0.2702$
<b>Female control</b>	Aa 1.5500 $\pm 0.3742$	Aa 1.6167 $\pm 0.3703$	Aa 1.6250 $\pm 0.3567$	Aa 1.6433 $\pm 0.2398$	Aa 1.6783 $\pm 0.2389$	Ab 1.7083 $\pm 0.2410$	Aa 1.2567 $\pm 0.2339$	Ab 1.7767 $\pm 0.2246$	Aa 1.8567 $\pm 0.2772$

$p < 0.01$  , N = number of animals . \*

Aa: Aa : no significant decrease

Aa : Ab : significant decrease

## DISCUSSION

The majority of absorbed toluene is metabolized by cytochrome P-450 (CYP) enzymes in the liver ,primarily to benzyl alcohol and is eventually eliminated in the urine as hippuric acid [ 14 ].

In our study the hitopathological changes in the lungs of males and females ,found thickening alveolar walls ,infiltration and proliferation of inflammatory cells , dilatation of bronchioles ,emphysema , fibrin into the alveolies and hemorrhage. Another on studies guinea pigs that exposed to TDI ( Toluene Di Isocyanate ) showed decrease respiration rate, pulmonary hypersensitivity ,the hitopathological effects including interstitial inflammation ,pleural thickening and peripheral lymphoid hyperplasia [15 ].Exposure to toluene at high levels can irritate the lungs ,extremely overexposure can cause pulmonary edema [16 ].

Changes into the tubules of the cortical area of the kidney were observed male rat ( 1.250 mg /kg ) and the majority in the ( 2.500 mg /kg ) and ( 5 mg /kg ) these changes included swollen tubules ,degeneration and necrosis of tubular epithelia , and presence of cast

in the tubular lumen [17 ]. In our study the histopathological changes in the livers and kidneys was degeneration and congested blood vessels and infiltration of inflammatory cells into the livers of males that to be in accordance with [18 ,19 ] chronic exposure to toluene cause damage in epithelial cells of tubules in kidney of rabbits .At very high level of exposure-such as might occur in an enclosed space or during a spill-toluene can injure the liver and kidney ,necrosis or fatty degeneration of heart, liver ,and adrenal [ 19 ].

Inhalation experiments with laboratory animals ,considerable amounts of toluene have been shown to be distributed to white adipose tissues , adrenal , kidney ,skin ,liver ,lung ,and brain [20 ,21 ] .The major pathway of toluene metabolism in both human and animals involves side chain oxidation by sequential action of cytochrome P-450 ,alcohol dehydrogenase and aldehyde dehydrogenase leading to benzoic acid which ,upon conjugation with glycine ,resulting in hippuric acid [ 22].

The uterus of females group shows , fibrosis of the uterus in the myometrium , oedema and epithelial cells in the muscles ,degeneration of cells lining the uterine glands with presence of red blood cells and inflammatory cells. There is no studies on histopathologic changes into the uterus but there was study shows toluene does not appear to be teratogenic in mice, rats, or rabbits, but embryotoxic / fetotoxic effects were seen in rats at a dose that was non-toxic for the dams exposed to toluene concentrations of 1000 mg/m<sup>3</sup> air, and spontaneous abortion occurred in rabbits exposed to 1000 mg/m<sup>3</sup> during the entire period of organogenesis. However, orally administered toluene was reported to be teratogenic in CD-1 mice. Exposure to 870 mg/kg body weight on days 6 – 15 significantly increased the incidence of cleft palate. A level of 430 mg/kg body weight was without effect[ 23 ] .

In a study by[ 24], New Zealand rabbits were exposed to 500 or 1000 mg toluene/m<sup>3</sup>, for 24 h/day, on days (6 – 20) of pregnancy.The toluene caused spontaneous abortions at 1000 mg/m<sup>3</sup> . Inhalation studies with mice and rats have shown that toluene has the potential to cause growth retardation and skeletal anomalies in the offspring of exposed dams. The potential for teratogenic effects appears limited. In mice exposed to toluene at concentrations of ( 400 -1000 ppm )a significant increase in the number of fetuses with extra ribs was observed [ 25- 27 ] .The offspring of rats exposed to ( 399 ppm ) showed decreased fetal weight and skeletal retardation [ 28 ].



## التغيرات النسيجية المرضية لاستنشاق التلوين في الأرانب

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### الخلاصة

صممت هذه الدراسة لتحديد تأثير التلوين على وظائف الأعضاء أو الصحة العامة للإناث والذكور . في هذه الدراسة تم استخدام ٢٤ أرنباً لكلاً للجنسين قسمت حيوانات الدراسة إلى أربعة مجاميع ، وكل مجموعة مؤلفة من ٦ أرانب . وتم تعريض الحيوانات إلى بخار التلوين المستنشاق بتركيز ( 500 ppm / ٢ ساعة / يوم ) يوميا لمدة شهران . مجاميع الحيوانات المعاملة بالتلوين لوحظ عليها نقصان في الوزن وقلة باستهلاك الغذاء . كما تم ملاحظة الدموع واللعاب خلال فترة الدراسة .

التغيرات النسيجية المرضية في الأعضاء ( الرئة ، الكبد ، الكلية ، والرحم ) لوحظ وجود اختلافات بسيطة بين الذكور والإناث. أما التغيرات النسيجية المرضية في رئت الذكور المعاملة فكانت الانتفاخ مع ترشح في الخلايا الالتهابية اللمفية في جدار الحويصلات الرئوية ونزف. أما التغيرات النسيجية المرضية في رئت الإناث المعاملة فكانت الانتفاخ أيضاً كما يوجد تثخن في جدار الحويصلات الرئوية ونزف ، بالإضافة لوجود مادة الليفين في الحويصلات وبعض القصيبات ، وزيادة في عدد أو تكاثر الخلايا الالتهابية .

التغيرات النسيجية المرضية الملاحظة في كلية الإناث المعاملة ، تنكس دهني وكذلك الانتفاخ الغيمي أو التنكس الغيمي . أما التغيرات النسيجية المرضية الملاحظة في كلية الذكور المعاملة فكانت التنكس الغيمي فقط . أما التغيرات النسيجية المرضية الملاحظة في كبد الإناث المعاملة فكانت تنكس مائي. أما بالنسبة للذكور فكان من الملاحظ وجود تنكس مائي ، احتقان الأوعية الدموية ، و ترشح في الخلايا الالتهابية (اللمفية) .

التغيرات النسيجية المرضية الملاحظة في رحم الإناث المعاملة لاحظنا وجود تليف ، وذمة ، وتنكس الخلايا المبطنة للغدد الرحمية مع وجود خلايا الم الحمراء والخلايا الالتهابية .

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