Histopathological effect of ethinylestradiol on lung of albino rats *(Ratus narwegecus)*

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Abstract

The present study was performed to investigate the histological lesions caused by ethinylestradiol, on albino rats lung. Three doses of ethinylestradiol were used 1, 2 and 3 mg/kg b.w./day for 4 weeks, the study were conducted on 20 rats divided into 4 groups, 3 groups were experimentally adminstrate orally ethinylestradiol and the last group was the control. After that the animals were killed by exsanguinations under chloroform anesthesia, and the lungs were fixed for histopathological studies.

Histological changes were observed in the lung manifested by focal attelactasis (failar of the lungs to expand fully), emphysema(the alveolar walls are a tropic and thin in places they have broken down to form spaces considerable larger than normal alveoli), congestion (accumulation of erythrocytes between the alveolar cells). Lymphatic infiltration(accumulation of large number of white blood cells between and inside alveolar sac), also showed accumulation of large number of lymphocytes around the bronchiole (peri-bronchial lymphocyte infiltration), Edema (accumulation of fluid), hemorrhage and hyperplasia smooth muscle in wall of pulmonary blood vessels.

Introduction

Oral contraceptives are highly efficient and easily administered drugs; however it must not be forgotten that they are composed of chemical substances which can be classified as potential carcinogens[1]. Ethinylestradiol (19-17 α - pregna 1, 3, 5 (10) – trien – 20 – yne – 3, 17 – diol), a synthetic steroidal estrogen, has long been used as a component of oral contraceptive and for the treatment of prostatic hypertrophy and cancer. There have been numerous reports dealing with toxicities attributed to ethinylestradiol in experimental animals which cause teratogenic effects on mouse fetus[2,3], and [4] show that estradiol has complex effects on the liver, it can lead to cholestasis. It affects the production of multiple proteins including lipoproteins, binding proteins and proteins responsible for blood clotting. Many observations suggested that 17 α- ethinylestradiol (synthetic estrogen) and 17 β- estradiol (endogenous estrogen) are likely to have similar effect in most tissues as in [5]. So estrogens are steroid hormones, produced by the ovaries, which exert effects on multiple target tissues including the brain [6]. Although, physiological importance of estrogens in male rodents was suggested by previous studies using the rat and mouse [7], on the other hand [8] suggested that estrogens might be important in lung development, physiology and carcinogenesis.

Also Andrea et al. [9] investigated the pathological phenotype of the lung in ERB (endogenous estrogen), they reported that lungs of both male and female mice are fibrotic with large regions of unexpanded alveoli. Jill, Breeze and Whceldon [10,11] Founded evidence of estrogen receptors in all five kinds of tumors from patients with non – small cell lung cancer, the most common variety of lung cancer, healthy lung cell rarely show estrogen receptors.

In the present study aim to investigate the histological alternative changes in albino rat lungs as aresult of ethinylestradiol administration.

Materials and methods:

-Animals and experimental design:-

Adult male (*Ratus narwegecus*) rats, weighting (320 – 350) gm. were obtained from the animal house of Science Education College / Salahaddin University. The animals were housed five per cage under controlled conditions of temperature (22ċ) and light (12 h light – 12 h dark cycle). They received standard diet and water *ad libitum.* Ethinylestradiol (19 -17 α- pergna -1,3,5 (10) trien -20- yne -3- 17 – diol) was dissolved in distilled water. The rats were divided into four groups (n= 5 per group), the first group was considered as control and received only distilled water, while the remaining three groups received 1,2 and 3 mg/kg b.w. of the drug respectively by gavages for (30) day.

-Histological studies:-

On the day after the final administration, all animals were weighted and killed by exsanguinations under chloroform anesthesia, and the lungs were prepared for histological examination. The lungs fixed in a formalin fixative (10% formalin with 90% distilled water), for at least 24 hours. The fixed lungs were dehydrated in ascending serious of alcohol, cleared in xylene and embedded in paraffin and consecutive sections (5-8)mm thick were obtained by a rotary microtome (Erma). And stained with (H & E) Harris Haematoxyline and Eosin,[12].

Result and discussion

Figure -1 illustrates the structures of 17 β- estradiol, the major endogenous estrogen produced by the ovary, and 17 α- ethinylestradiol which is the estrogen used most commonly in oral contraceptives [5]. On the other hand ERB is the predominant estrogen receptor in the lung as shown in [13,8]. However, there is very little information about the mechanisms of estrogen action and estrogen receptor expression in the lung, the lung has been estimated to consist of 40 or more

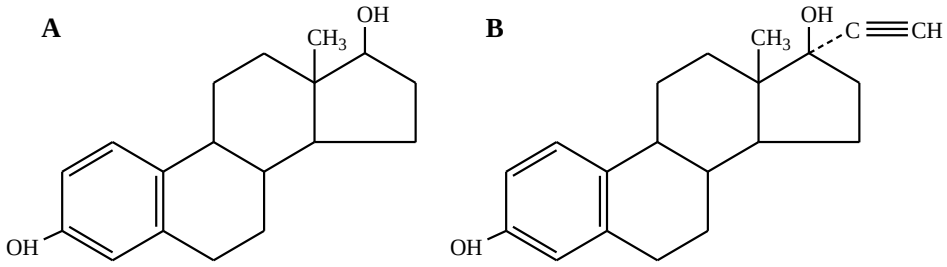
different cell types as in [14,7], for this reason we started our investigation of a potential estrogen action in the lung.

Our results showed that ethinylestradiol depending on concentration affected on the lungs. Figure -2 section from normal rat lung showing normal alveoli and alveolar sac. While figure -3 showed histological changes in the lung of exposed rat to orally administration of ethinylestradiol (first concentration) showed focal attelactasis (failure of the lungs to expand fully), while after second concentration exposure the lung become emphysema (the alveolar walls are atrophic and thin in places they have broken down to form spaces considerable larger than normal alveoli as in figure -4.

Also figure -5 showed accumulation of erythrocytes (congestion) between the alveolar cells, on the other hands after exposed to third concentration in figure -6 showed lymphatic infiltration (accumulation of white blood cells between and inside alveolar sac), figure -7 showing peri-bronchial lymphocyte infiltration (malignant lymphoma), edema figure -8 (accumulation of fluid), hemorrhage and hyper plastic smooth muscle in wall of pylmonary blood vessels figure -9.

The vital function of the lung is to provide gas – exchange surface to supply the organisms needs for oxygen uptake and carbon dioxide elimination, ERB is the predominant estrogen receptor in the lung [8] . There are numerous reports supported our results that ethinylestradiol affected on lung as in [15] demonstrated that the lung volume become reduced after female mice exposed to estrogen, also reported to have fewer alveoli and attributed the lung phenotype to some defect of the extracellular matrix compositions.[16] cleared that estradiol has proinflammatory actions on the allergic female rat lung response as observed by elevated number of eosinophils, mononuclear cells and neutrophils.

The mechanism of action of estradiol as cleared by [17,9] showed that its enters cells freely and interact with acytoplasmic target cell receptor, when the estrogen receptor has bound its ligand it can enter the nucleus of the target cell, and regulate gene transcription which leads to formation of messenger RNA. The mRNA interacts with ribosome's to produce specific proteins that express the effect of estrodiol upon the target cell. On the other hand [18] demonstrated that 17 β- estradiol significantly attenuated inflammatory response as measured by histological examination and exudate production. And the mechanism by which estradiol has benefical effects in the model of inflammation are unclear. The same authers cleared that in hormonally treated rats there is a decrease in polymorphonuclear cells migration as shown by counting and myeloperoxidase measurement. In addition, ethinylestradiol treatment opposes to induced high lipid per oxidation.



17 β - Estradiol

17 α - Ethinylestradiol

Fig (1): Structures of (A) endogenous estrogen 17 β – E2 and (B) Ethinylestradoil 17α - EE

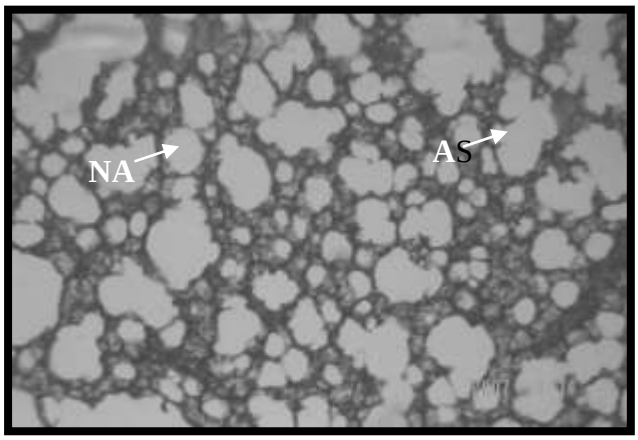


Fig (2): Section from normal rat lung showing Normal Alveoli (NA) and Alveolar Sacs (AS) 100X (H & E)

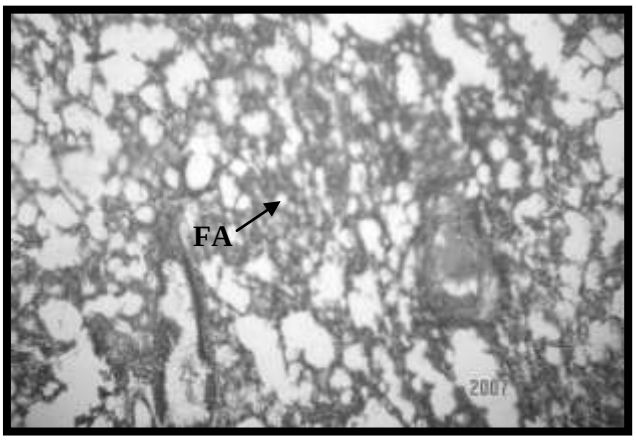


Fig (3): Section from treated rat lung showing Focal Attelactasis (FA) after exposed to (1 mg) ethinylestradiol (100x) (H& E)

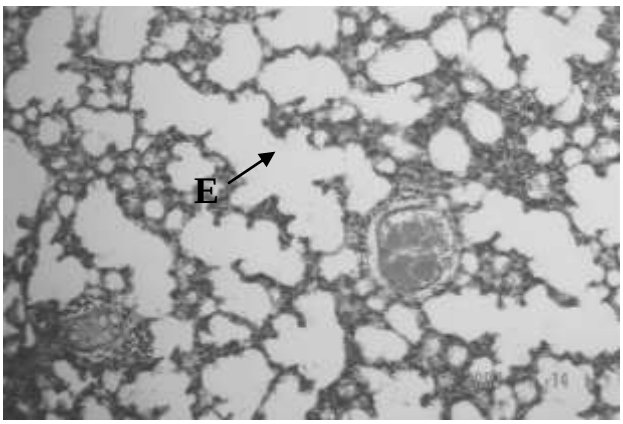


Fig (4): Section from treated rat lung with (2 mg) ethinylestradiol showing Emphysema (E) (250X) (H& E)

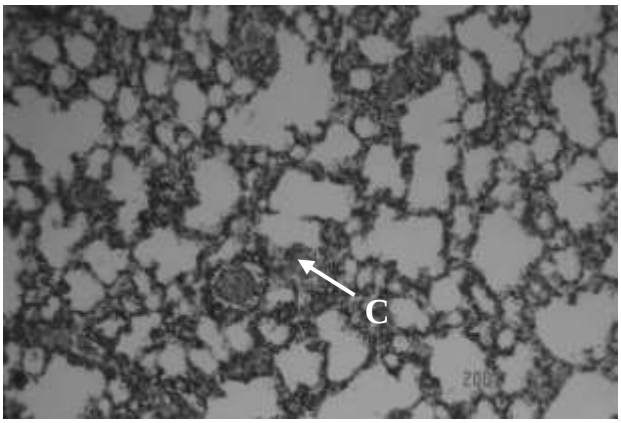


Fig (5): Section from treated rat lung with (2 mg) ethinylestradiol showing congestion (C) accumulation of erythrocytes between the alveolar cells (100x) (H& E)

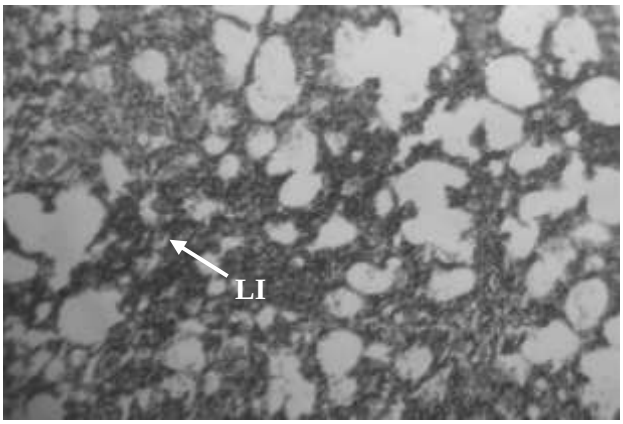


Fig (6): Section from treated rat lung showing Lymphatic Infiltration (LI) exposed to (3 mg) (100x) (H& E)

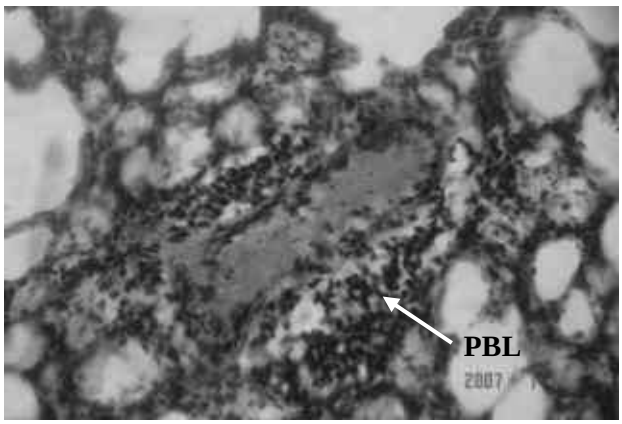


Fig (7): Section from treated rat lung showing peri – branchial lymphocyte infiltration (PBL) exposed to (3 mg) (400x) (H& E)

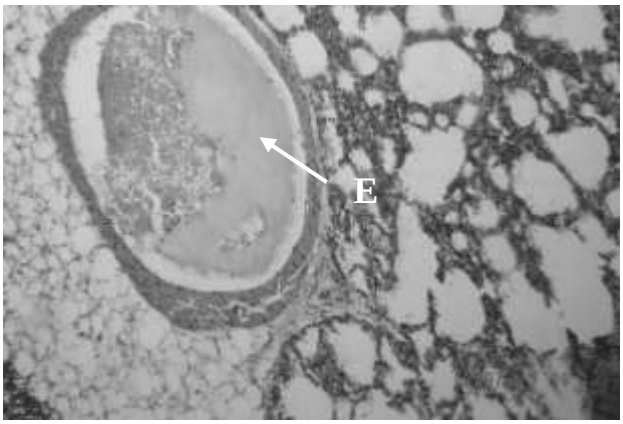


Fig (8): Section from treated rat lung showing Edema (E) exposed to (3 mg) (250x) (H& E)

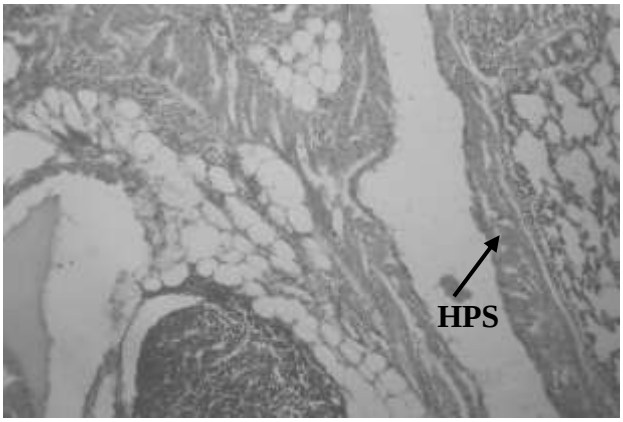


Fig (9): Section from treated rat lung showing Hyper Plastic Smooth (HPS) muscle in wall of pulmonary blood vessels exposed to (3 mg)(100x) (H& E)

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التأثير الامراضي النسيجي لـ Ethinylestradiol على رئة الجرذان البيضاء *(Ratus narwegecus)*

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الملخص

تضمنت الدراسة الحالية تقييم التأثير النسيجي ل ethinylestradiol في رئة الجرذان البيضاء، استخدم ثلاث جرعات ٣،٢،١ ملغم \ كغم وزن الجسم \ يوم من مادة ethinylestradiol لمدة أربعة أسابيع، تم تقسيم عشرون جرذا إلى أربعة مجاميع تعرضت ثلاث مجاميع الأولى لمادة ethinylestradiol عن طريق التجريع، أما المجموعة الرابعة فقد استخدمت كمجموعة تحكم ثم تم تخدير الحيوانات بواسطة مادة الكلوروفورم واخذ مقاطع من الرئة لدراسة التغييرات النسيجية والمرضية.

أوضحت التغييرات النسيجية حصول (فشل الرئة في الاتساع) وكسر جدران الأكياس الحوصلية لتكوين مساحات كبيرة مقارنة بالمساحة الطبيعية للجدران الحويصلة والاحتقان الدموي (تجمع كريات الدم الحمر بين الخلايا الحوصلية وارتشاح التهابي (تجمع أعداد كبيرة من كريات الدم البيض بين وداخل الأكياس الحوصلية وأظهرت النتائج حدوث حالة الخزب ونزف الدم في جدران الأوعية الدموية للرئة.