Effect of ethinylestradiol on testis of albino rats
(Ratus narwegecus)

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Abstract:
The present study was conducted to clarify the histological effect of ethinylestradiol, on albino rat testis. The albino rats were administrated three oral dose of ethinylestradiol 1,2and3 mg/kg b.w./day for 4 weeks. After that the tests were fixed for histological studies. Histological changes were observed in the testis, manifested by (degenerative changes in spermatogenesis, disruption in normal epithelial organization, decreased number of elongated sperm, depletion in germinal layer, dilatation of interstitial space, existing vacuoles in certain regions of interstitial spaces and present of giant multinucleated cell. Also results indicated that ethinylestradiol has affected on the body and testis weight. We attribute the testis toxicity during ethinylestradiol exposure to the suppression of testicular testosterone levels.

Introduction:
Ethinylestradiol (19-17 α-pregna-1,3,5 (10)- trien-20 -yene-3,17-diol), a synthetic steroidal estrogen, has long been used as a component of oral contraceptives and for the treatment of prostatic hypertrophy and cancer. There have been numerous reports dealing with toxicities attributed to ethinylestradiol in experimental animals, teratogenic effects on mouse fetus [1], anemia, edematous swelling of liver cells, hypertrophy of the adrenal gland and pituitary, and depression of gonadal organs in rats [2].

Concerning the hepatocarcinogenesis in rats and humans [3 and 4], regarding to the testicular toxicity in rats, ethinylestradiol has been reported to evoke sterility as a result of decreased testicular sperm counts, and atrophy of seminiferous tubules [5]. Furthermore, sperm motion parameters such as the percentage of motile sperm, velocity, and amplitude of the lateral head displacement, were decreased when administrated ethinylestradiol at 10 mg/kg to rats for 1 week [6].

In the present study we try to investigate the histological alternation in albino rat testis in response to ethinylestradiol administration.

Materials and methods:
Animals and experimental design:
Adult male (Ratus narwegecus) rats, weighing (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 (22°C) and light (12h light:12h dark cycle). They received standard diet and water ad libitum. Ethinylestradiol (19-17 α-pregna-1,3,5 (10)- trien-20 -yene-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 1,2and3 mg of the drug respectively by gavages for (30) day.

Histological studies:
On the day after the final administration, all animals were weighed and killed by exsanguinations under chloroform anesthesia, and the testis were weighed then prepared for histological examination.

The testis fixed in a formalin fixative (10 % formalin with 90% distilled water), for at least 24 hours. The fixed testis were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin and consecutive sections (5-8) µm thick were obtained by a rotary microtome (Erma), and stained with (H and E) Harris Haematoxylin and Eosin, [7].

Results and discussion:
Testicular section showed normal spermatogenesis in control groups Fig(1). However, after ethinylestradiol treatment, different degrees of degenerative changes in spermatogenesis were observed. The damage that occurred was variable. At the lowest dose of ethinylestradiol (1mg/kg/day) spermatogenesis was qualitatively normal, although some tubules appeared atrophied Fig(3a) and showed disruption in normal epithelial organization. Moreover, tubular damage increased with the increasing dose of ethinylestradiol. At the highest dose of ethinylestradiol testicular changes were characterized by decrease in the number of elongated spermatid, degenerated germ cell Fig(2).

There were also vacuoles in certain region of interstitial spaces Fig(5), suppression of spermatozoa, depletion in germinal layer, dilatation of interstitial space Fig(3b and c) and appearance of giant multinucleated of cells Fig (4).

Histological data indicated that ethinylestradiol treatment inhibits spermatogenesis in certain areas of the seminiferous epithelium. Seminal results were reported by [8] who suggested that the absence of the multiple steps of germ cell development resulted in the ability to distinguish complete stage profiles normally associated with the seminiferous epithelial cycles. The results of [9] showed testicular damage of rats when treated with different doses of oestradiol. Also [10] cleared that ethinylestradiol induced testicular damage when exposed to rat by increasing testicular testosterone level, in which is thought to suppress testosterone production in ledig cells which was histologically reflected as atrophy. The mechanism by which ethinylestradiol induced testicular testosterone cleared by [11] who expected that ethinylestradiol decreased serum testosterone below the minimum detectable levels and testicular testosterone levels declined about 2.5% of the control level. A serum testosterone level is maintained by an endocrine control system characterized by the negative feedback in the hypothalamus-pituitary-testis-axis. The gonadotropes releasing hormone (GnRH) secret pulsative from the hypothalamus and reaches the anterior pituitary through the pituitary portal vein. Then luteinizing hormone (LH)
and follicle stimulated hormone (FSH) are synthesized in and secreted from gonadotropic cells of the pituitary. LH combines with the receptors cAMP and subsequently testosterone. Testosterone is secreted from leydig cells into blood testosterone level, and by paracrine action reaches germ cells in seminiferous tubules causing stimulation of spermatogenesis. Blood testosterone is metabolized to estradiol 17B in brain. Increased serum estradiol 17B level inhibits the hypothalamus to produce GnRH by the feedback system leading to decrease blood testosterone level. Ethynylestradiol has the same action as that of estradiol 17B and LH, FSH, and testosterone [12] as the case with other synthetic estrogen, hexaestrol, diethylstilbisterol, fosfestrol, allylestrenol, and mestranol [13,14and15] this could be due to the suppression of GnRH secretion from the hypothalamus by the negative feedback, and eventually decreased testosterone production in testicular leydig cells could induce hypospermatogenesis. As another factor contributing to ethinylestradiol testicular toxicity, severe decreases in body weight were seen depending on concentration in all animal treated. The data in Table (1) revealed that the weight of body and testis of rats affected significantly (p≥0.01) by different concentration of ethinylestradiol.

The mean values of data showed that the highest weight of body: 33.40 gm and testis: 1.87 were recorded from control, whereas the lowest weight (266.20 and 1.04) of above parameters produced in case of high concentration. In previous studies, suppression of body weight gain and food intake have been reported, and estrogen explained to diminish efficiency of ingested protein and depress growth hormone secretion from the pituitary gland. Also [16] reported that ethenylestradiol affected on body weight. Depending on blood testosterone level, weight has been reduced in rats received 0.2, 0.3, 1 mg/kg/day of ethenylestradiol. On the other hands, [17] cleared that malnutrition itself could induce hypospermatogenesis. Restricted feeding has also been recognized to induce hypospermatogenesis when body weight of rats were reduced to approximately 50% those of the control rats. Histologically degeneration of pacheten spermatocytes in stage VII was observed [18].

**Table (1):** Mean values for the effect of ethinylestradiol treatment body and testes weights in rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Testes weight gm</th>
<th>body weight gm</th>
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</thead>
<tbody>
<tr>
<td>C0</td>
<td>1.87</td>
<td>331.40</td>
</tr>
<tr>
<td>C1</td>
<td>1.87</td>
<td>320.60</td>
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<td>C2</td>
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<tr>
<td>C3</td>
<td>1.04</td>
<td>266.20</td>
</tr>
<tr>
<td>LSD(0.01)</td>
<td>0.21</td>
<td>12.58</td>
</tr>
</tbody>
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**Fig (1):** Section from normal rat testis showing normal seminiferous tubules contained spermatozoa, the tails (T) directed toward lumen and the heads (H) towards aggregation of spermatids X20.

**Fig (2):** Section from treated rat testis with (3 mg) ethinylestradiol showing

1- degenerated germ cell (DG)
2- suppression of sperm (SS) X20.
Fig (3): Section from treated rat testis showing 1- depletion in germinal layer (DG) 2- dilatation of interstitial space(DIC) (a) after exposed to (1mg) ethinylestradiol,(b )after (2mg) ethinylestradiol, (c) after (3mg) ethinylestradiol X10.

Fig (4): Section in rat testis showing giant multinucleated cell (GM) after (2mg) ethinylestradiol X40.

Fig (5): Section in rat testis showing vacuolated germ cells(V) with (2mg) ethinylestradiol X20.

References:
9- Sharp R., Doogan D., Cooper I. Factors determining whether the direct effects of an LHRH agonist on leydig cell function in vivo are stimulatory or inhibitory. *Molecular and Cellular Endocrinology* 32:57-71(1983).

**Ratus narwegecus**

تأثير ايثنايل استرادايول على الجرذان البيض

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الملخص:

تتضمن الدراسة الحالية تقييم التأثير التنموي لمادة Ethinyl estradiol في خصي نكرالجرذ الأبيض حيث جربت الحيوانات بمادة Ethinyl estradiol لثلاث جرعات 1 و 2 و 3 مليمغ/زن الحيوان/يوم عن طريق الفم لمدة أربعة أسابيع. تم تقييم تأثير التسمم الخصوي أثناء التعرض ل Ethinyl estradiol إلى كيت مستويات الشحم الخصوي.