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17β-estradiol residue determined in minced meat and it's Carcinogencity in mice

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Abstract:

The main objective of this research to study carcinogenic effects of 17β-estradiol in mice by studying on haematological parameters (Hb, PCV, RBC, WBC and DWBC), mitotic index and histopathological changes of ovary, uterus and mammary gland. The following results which obtained from the current research:

The effects of 17β-estradiol for six months on blood picture had no-significant differences on the blood haemoglobin (Hb) (11.55 and 11.69 gm/dl), total number of red blood cells (RBC) counts in the peripheral blood. RBC counts were 7.48 and 6.98 × 106/mm3 for the two groups respectively. Basophiles percent (3.40 and 3.54%) also (8.68 and 8.09%) for Monocytes percent for the two experimental groups respectively. Significant decrease (P<0.05) in PCV% were noticed between the control and treated group (37.40and 31.28 %) respectively, and Lymphocyte percent (49.00 and 46.03%). Also significant increases (P<0.05) were observed in the WBC count between the control and the second group (7.05 and 9.43 109/l), and the Neutrophil percent (35.52 and 37.35%) respectively, also significant increases (P<0.05) in the Eosinophil percent (3.40 and 4.99 %) respectively. The results of carcinogenic effects on cytogenic study showed a significant increase (P< 0.05) in mitotic index for mice treated with17β-estradiol in comparison to control group. While the histopathological changes, in ovary: In mice treated with 17β-estradiol revealed immature development of follicles that primordail and primary follicles can be detect while secondary follicle was noted without ova and no griffin follicle can be seen. The histopathological study of uterus: In mice treated with 17β-estradiol showed dilatation of endometrial glands with hyperplasia of epithelial cells lining uterus and there is compact hypercellular stroma. While the histopathological changes, in mammary gland: The mammary gland of treated mice with 17-β estradiol showed pleomorphic hyperchromatic malignant cells in addition somewhere arranged as glandular structure, but the gross appearance of mammary gland adenocarinoma gives enlargement with irregular shape.

Keywords: *17β-estradiol, Carcinogencity*

Introduction:

Hormones are vital in normal development, maturation and physiological functioning of many vital organs and processes in the body; however, like any other chemicals of natural or synthetic origin, hormones can be toxic to living organisms under certain circumstances (1). Growth-promoting implants offer beef cattle producers a safe and effective way to increase calf weight gains and increase production of muscle tissue and often reduce body fat production. This result is significant improvements in both growth rate and feed efficiency (2). There are six hormones approved for use in beef production in more than 30 countries. Three of these are natural, three synthetic. The three natural hormones (testosterone, estradiol, and progesterone) are a natural part of all mammalian physiology. The three synthetic growth enhancing hormones are melengestrol acetate (MGA), trenbolone acetate (TBA), and zeranol (3, 4, 5). Growth promoting hormones typically are administered through a small pellet (called an implant) that is placed under the skin on the back of an animal's ear (6), but some can be administered through the animal feed (MGA, unlike the other GPHs, is administered via the diet as a feed additive) (7). The health concerns associated with hormonal compounds used as growth promotants (and also as therapeutic agents) are their carcinogenic and endocrine-disrupting potentials. By virtue of their normal biochemi-

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cal action, low concentrations of steroid hormones (nM) bind to and activate their intracellular receptors, which interact with hormone response elements in DNA, leading to the transcription of genes that induce cell proliferation and growth. Therefore a hormonal substance could promote carcinogenicity in hormone-sensitive tissues through such a proliferative mechanism (8,7).The SCVPH (9, 10) concluded that the risk associated with the consumption of meat from hormone-treated cattle may be greater than previously thought (10), it indicated that there was a significant body of evidence suggesting that 17β-estradiol should be considered a complete carcinogen.

Material and Methods:

Collection of minced meat samples:

Thirty samples of imported minced meat (Brasilian and Indian origin and different companies) were collected from markets and super markets of Baghdad province.

Determination of residual 17β-estradiol in minced meat by HPLC: The analysis was performed on an Agilent 1200 Series HPLC with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 (5 µm 250 mm × 4.6 mm id, p/n 959990-902). An Agilent 0.45-µm PTFE Premium Syringe Filter (p/n 5185-5836) was used to filter the sample solution before HPLC. Hormone standards from Sigma Company, the SPE cartridges were Agilent SampliQ OPT (3 mL, 60 mg, p/n 5982-3036).

Sample preparation, separation and SPE Purification: According to the procedure by (11).

Laboratory Animals: Forty female albino BALB/C mice were used and maintained at the animal house laboratory in Iraqi center of cancer and medical genetic researches. Mice were divided in two groups each of 20 female. The first group was untreated (control) and the second group treated with 17- β estradiol hormone (420 ppb) by drinking water for 6 month.

Blood Collection: The blood samples were collected at the end of experiment from the groups according to (12).

Cytogenetic study (Mitotic Index): The sacrificed mice from each group of carcinogenic study at the end of experiment were used for measuring mitotic index detected according to Allen method (13), used femur bone marrow.

Preparation of Tissues for Histopathological Studies: Were done according to (14).

Statistical Analysis: Statistical package for social sciences (SPSS) software version 16 was used for performing statistical analysis (15).

Results: The levels of 17β-estradiol hormone residues in (brasilian and indian) samples are listed in (Table 1). The data clearly demonstrated that the levels of 17β-estradiol hormonal residues were significantly higher (P< 0.05) in Indian 495.0 ppb than Brasilian 310.6 ppb.

Table 1: HPLC concentration levels of 17β-estradiol (ppb) in imported minced meats.

No. of samples	country	17β -estradiol	
		R	M ± SE
15	India	397.9-578.4	**a** 151.78 495.0±
15	Brasil	182.0-392.8	**b** 310.6±187.76

Different letter within the same column are significantly different (p< 0.05).
R = Range M ± SE = Mean ± Stander Error

The results that presented in (Table 2) declared that administration of estradiol 17-β for six months had no-significant differences on the blood hemoglobin (Hb) (11.55 and 11.69 gm/dl), total number of red blood cells (RBC) counts in the peripheral blood. RBC counts were 7.48 and 6.98 × 106/mm3 for the two groups respectively. Basophiles percent (3.40 and 3.54%) also (8.68 and 8.09%) for Monocytes percent for the two experimental groups respectively. Significant decrease (P<0.05) in PCV% were noticed between the control and treated group (37.40and 31.28 %) respectively, and Lymphocyte percent (49.00 and 46.03%). Also significant increases (P<0.05) were observed in the WBC count between the control and the second group (7.05 and 9.43 109/l), and the Neutrophil percent (35.52 and 37.35%) respectively, also significant increases (P<0.05) in the Eosinophil percent (3.40 and 4.99 %) respectively.

Table 2: Effect of 17-β estradiol on blood pictures in mice (M ± SE)

Parameter	Groups	
	Control	Treatment
Hb gm/dl	[a] 0.56 11.55±	[a] 0.30 ± 11.69
% PCV	[a] 0.31 37.40±	[b] 1.00 ± 31.28
RBC 10^6/mm^3	[a] 0.24 ± 7.48	[a] 0.19 ± 6.98
WBCs 10^9/l	[a] 0.1 ± 7.05	[b] 0.20 ± 9.43
% Lymphocyte	[a] 0.12 ± 49.00	[b] ±0.40 46.03
% Neutrophil	[a] 0.22 ± 35.52	[b] 0.31 ± 37.35
% Monocytes	[a] 0.16 ± 8.68	[a] 0.70 ± 8.09
% Eosinophil	[a] 0.06 ± 3.40	[b] 0.40 ± 4.99
% Basophils	[a] 0.02 ± 3.40	[a] 0.08 ± 3.54

Different letter within the same row are significantly different ($p< 0.05$).

The results of (MI) mitotic index in mice showed the highest mitotic index value was demonstrated statistically ($P<0.05$) in mice treated 17-β estradiol (Fig.1) compared to mice control (Table 3).

Table 3: Mitotic index (%) in experimental mice (mean ± SE).

Mitotic index	Groups	
	Control	Treatment
	[a] 0.03 ± 0.7	[b] 0.02 ± 1.6

Different letter within the same row are significantly different ($p< 0.05$).

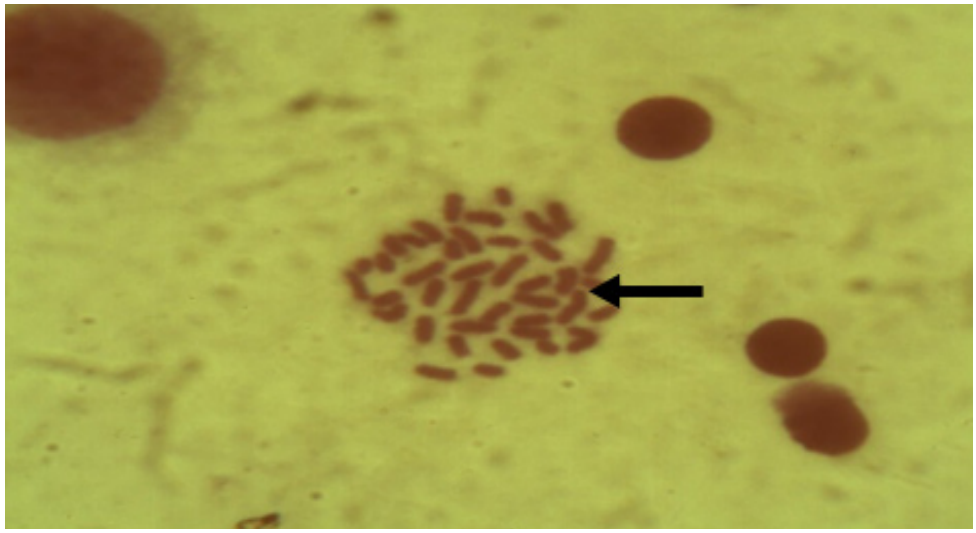

Figure 1: Bone marrow cells showing cell in metaphase (↑) X1000

The histopathological changes of treated normal mice with the 17 β-estradiol: In ovary for mice treated with 17β-estradiol revealed immature development of follicles that primordail and primary follicles can be detect while secondary follicle was noted without ova (Fig. 2) and no griffin follicle can be seen. The control (untreated) group shows no changes in ovary (Fig. 3).

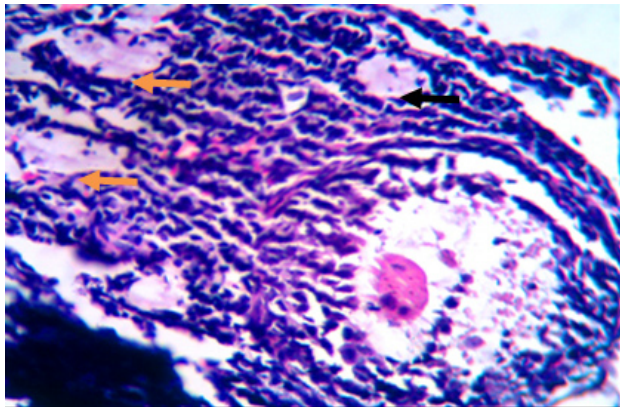

Figure 2: Section of ovary for mouse treated with 17β-estradiol show ill defined ova seen inside the secondary follicle (↑) and primary follicle (↑) (H&E 200 X).

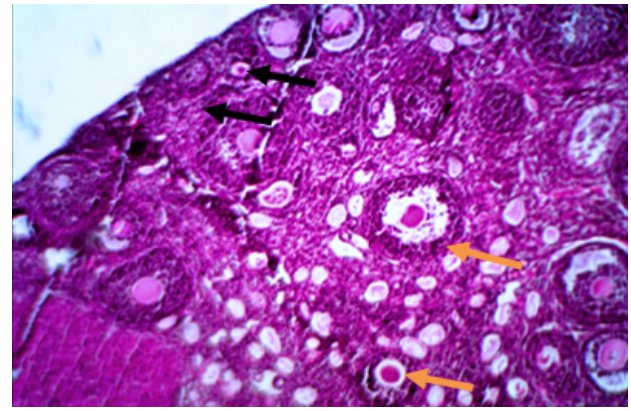

Figure 3: Section of ovary for mouse untreated (control) showing different mature (↑) and immature follicles (↑) (H&E 100X).

While the histopathological changes of uterus: The section of uterus for mice treated with 17-β estradiol, showed dilatation of endometrial glands with hyperplasia of epithelial cells lining uterus and there is compact hypercellular stroma (Fig. 4 and Fig. 5). The uterus of control group showed no changes (Fig. 6).

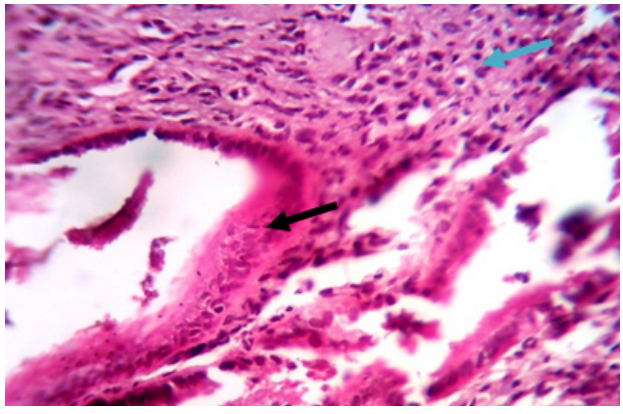

Figure 4: Section of uterus for mouse treated with 17β-estradiol show endometrial glandular epithelial hyperplasia (↑) and compact hypercellular stroma (↑) (H&E 200 X).

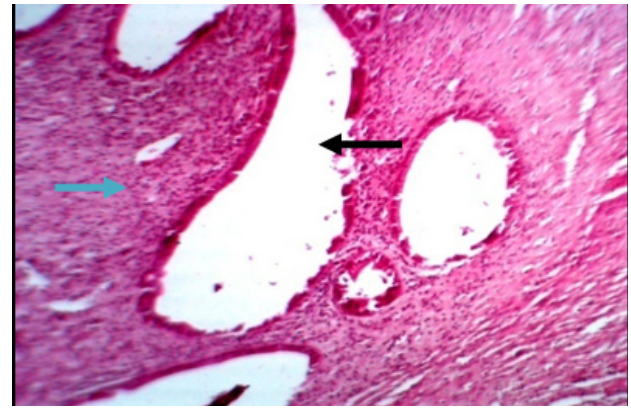

Figure 5: Section of uterus for mouse treated with 17β-estradiol show endometrial glandular dilation (↑) and compact hypercellular stroma (↑) (H&E 100 X).

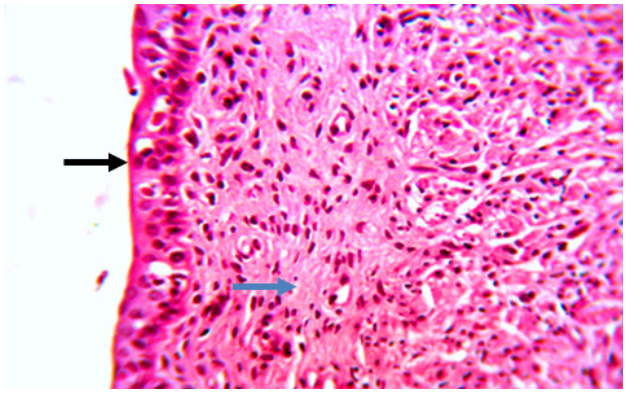

Figure 6: Section of uterus for mouse untreated (control) showing normal tissue, endometrial layer (↑) consist of columnar epithelial cell lining glandular structure; myometrial consist of smooth muscle layer (↑) (H&E 400X).

Effects 17-β estradiol on mammary gland: The gross appearance of mammary gland adenocarinoma gives enlargement with irregular shape (Fig. 7).

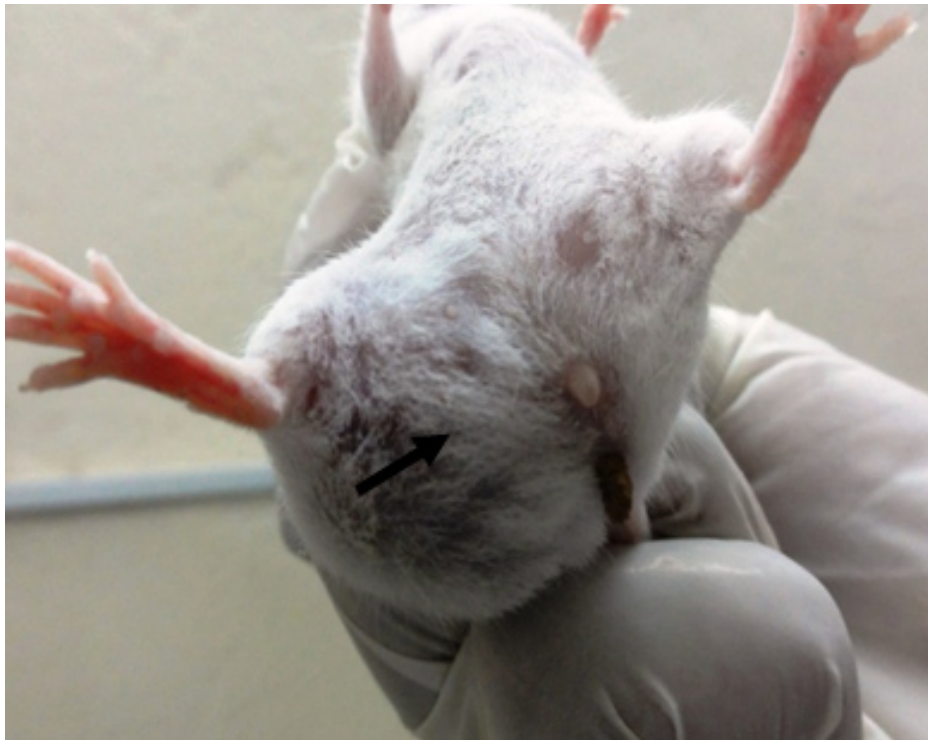

Figure 7: Adenocarinoma of mice treated with 17β-estradiol showing enlargement and irregular mass (⬆).

The histopathological changes of mammary gland of treated mice with 17-β estradiol showed pleomorphic hyperchromatic malignant cells in addition somewhere arranged as glandular structure (Fig. 8 and Fig. 9). All these changes give indication for malignant adenocarcinoma of mammary gland as a result of treated with 17-β estradiol.

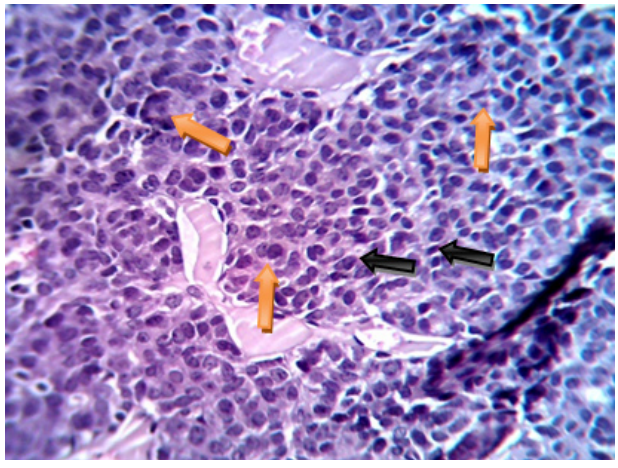

Figure 8: Section of mammary gland for mouse treated with 17β-estradiol show sheets pleomorphic malignant cells (⬆) and glandular structure (⬆) (H&E 200 X).

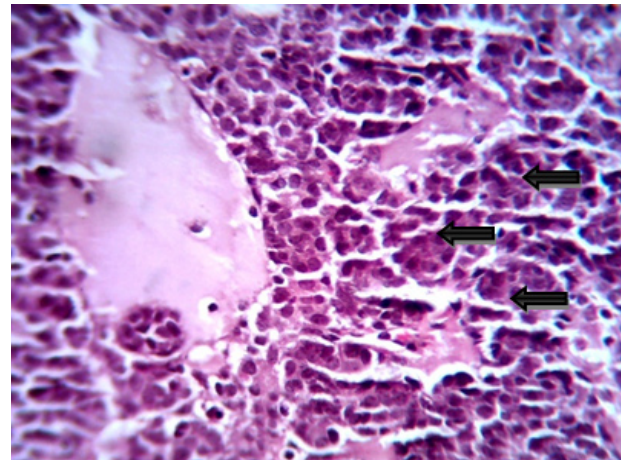

Figure 9: Section of mammary gland for mouse treated with 17β-estradiol show malignant glandular structure (⬆) (H&E 200 X).

Discussion:

The mean hormonal residue levels in imported samples reported in this research were higher than the acceptable hormone levels value for estradiol of 0.05 µg/kg body weight /day as assessed by (16). This can be attributed to:

Multi-implanted and re-implanted animals, this implantation process increase the mean of 17β- estradiol concentration by induces an accumulation of the free form of 17β-estradiol, and their concentration increased when overdose was applied (16). (17) founds that in multi-implanted and re-implanted of steers, that the concentration of 17β- estradiol was 0.2% for untreated animals, and 1.3% for treated animals (single implant), while the 17β- estradiol reached a mean of 3.9% for the animals received two implants and to a mean of 4.7% for those injected with four implants. (18), who found that the levels of 17β-estradiol hormonal residues were largely due to the fat content in meat (fatty acid esters) which is the lipoidal esters. Fats account for approximately 50% of the total 17β-estradiol concentration in untreated steers (69 ng/ kg fat) compared to muscle (32 ng/ kg) and in a single implant steers (158 ng/ kg fat) than in muscle (87 ng/ kg muscle). Minced meat may come from site of implantation (ears) which has not been discarded after slaughter (19). The total residues of hormones in the outer regions of the areas are 300 times lower than in the inner regions, and did not exceed 2µg. The hydrolysis of steroids was negligible in residual implants, but relevant in the surrounding tissue areas (20). When hormonal implant injected into different parts of the animal body (not discarded after slaughter) can enter human food (21), further, when the ears of the treated animals are not discarded after slaughter, milligrams of hormone residues potentially enter human food, and by consuming complete implantation sites, the consumer ingests higher amounts that can have an acute effects on consumer health (22). Decreased fertility with maternal aging has been well documented in animals (23) due to the fact that the older animals did not show a consistent pattern of steroid hormone concentrations, but eventually decrease fertility with advanced age (24, 25). The mean life expectancy in cattle was 19 yr and 55% of the herd was infertile by 13 yr of age with serum concentration of estradiol of 19.2 ng/ ml (26). (27) noticed that the estradiol concentrations were higher in cow at estrus cycle period. Increase in follicular size was associated with an increase in estradiol concentration and a decrease in progesterone concentration. Minced meat may be prepared from animals treated with estrogen as medicine as in estrus-synchronization programs by veterinarians (28, 29).

The non-significant differences which obtained for the red blood cells count and hemoglobin, because of estradiol 17-β as steroidal hormone secreted by the ovaries which enhance lipid metabolism and increase sedimentation rate of RBC and unchanged the count of RBC cells (30). (31, 32), those found unchanged in total RBC count in broiler chicken; non-significant difference in hemoglobin concentration and non-significance in RBC number and hemodilution. Conversely, the amount of PCV% was reduced significantly during treatment, this reduction may be due to the decrease of blood cell level. In bone marrow the increasing in the proportion of immature RBC cells, may give conclusion that continuously increasing in estradiol concentrations as an inducer of erythropoisis proliferation and differentiation arrest, resulted in increasing production of RBC (partially immature) , these initial results were almost comparable to that reported by (33).The increasing of WBCs count in mice treated with estradiol 17- β may be due to the temporary increasing of estrogen (34).The results showed that estrogen down-regulate the expression of adhesion and chemokine molecules in response to inflammation promotes in various experimental system. Functional results showed that estrogen treatment attenuates recruitment and adhesion of leukocytes to the endothelium induced by inflammation promoters offering a possible mechanism by which estrogen exert an anti inflammatory effect, these effects of estrogens due to focusing on the interaction of monocytes with the vascular endothelium (35).

The non-significant increase in basophiles during treatment occurred (36, 37, 38, 39) explain the fact that the effect of progesterone during pregnancy when compared with the follicular phase of normal ovarian cycle, but this non increase in basophiles may due to the negative feedback mechanism of the estrogen to the pituitary gland and cause to decrease the secretion of estrogen. The number of monocytes was decreased during estradiol treatment and this coordinate with the result of (40, 35).Such decrease and then return to the normal range after administration leads to the suggestion that estradiol inhibit the monocytes chemoatractant protein-1- induced monocytes migration through non-genomic estrogen receptor alpha. This may explain one of the anti-atherosclerotic effects of estradiol on vasculature. Increasing the mitotic index and increasing nuclear instabilities were detected accompanying transformation and tumorigenesis induced in the MCF-10F cells by 17 –β estradiol (41, 42, 43). Also the mitotic abnormalities contributing to nuclear disturbances are expected to become more representative with the 17 β-estradiol induced transformation/ tumorigenesis progress (44) and in view of a previous report that estrogen treatment increases the number and mitotic activity of erythroid precursors in mouse spleen and bone marrow (45). The histopathological changes in ovary, uterus and mammary gland occurred due to that the estrogen exerts its effect through two receptors, ERα and ERβ. A number of studies have addressed the expression of both isoforms in clinical samples and their functions in cell line models. ERβ is highly expressed throughout the normal ovary, including granulosa cells, theca cells, corpora lutea, oocytes, as well as cultures of primary ovarian surface epithelial cells, in the mammary epithelium and the mammary stroma and others organs (46, 47, 48, 49). However, its expression is progressively lost during cancer development and progression (50, 51, 52). While this loss

has been associated with loss at the genetic level, there is increasing evidence that lower expression of ERβ can also result from epigenetic changes, namely hypermethylation of its promoter (53, 54, 55). A recent study showed nuclear localization of ERβ in normal ovarian tissue, but cytoplasmic localization in the tumor tissue (56). In contrast, ERα expression is maintained, or even increased, in a subset of tumors (52). As a result, there is an increase in the ERα/ERβ ratio with malignant progression of the tumor (57). A number of studies clearly showed that estrogen treatment exerts pro-proliferative action (58, 59). Estrogen was originally believed to cause cancer by helping cells proliferate. After the hormone binds to its receptors in a cell, it turns on hormone-responsive genes that promote DNA synthesis and cell proliferation. If a cell happens to have cancer-causing mutations, those cells will also proliferate and have a chance to grow into tumors (60, 61).

So it is hypothesized that estrogen metabolism may play a key role in estrogen-induced cancers because different estrogens differ in how they're broken down in the cell" (60, 62). The cell uses a series of reactions to rid itself of estrogen. In metabolizing carcinogenic estrogens, the reactions produce intermediates capable of producing oxygen radicals that can damage the cell's fats, proteins, and DNA. Unrepaired DNA damage can turn into a mutation, which can later promote cancer (63, 64). To explaine if cancer-causing estrogens need oxygen radicals to produce tumors, (65) implanted pellets of the hormone in hamsters that are susceptible to estrogen-induced kidney cancer. This model is widely used as an animal model of hormonal cancer. As expected, when the carcinogenic 17β-estradiol was used, nearly all hamsters with the pellets developed cancer within seven months. Estradiol-17β promotes cells proliferation and produce oxygen radicals when metabolized by the cell (66).

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تحديد متبقيات -17 بيتا استرادايول باللحوم المثرومة و تاثيرها المسرطن في الفئران

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الخلاصة:

استهدفت البحث دراسة التأثير المسرطن لل -17 بيتا استراديول في اناث الفئران من خلال دراسة بعض المؤشرات: الصورة الدموية (كريات الدم الحمراء , خضاب الدم , حجم الخلايا المرصوصة , كريات الدم البيض والعد التفريقي لكريات الدم البيض), التأثيرات الخلوية (معامل الانقسام الخلوي) ودراسة التغيرات المرضية النسيجية لكل من (المبيض , الرحم والغدة اللبنية).

أظهر التأثير المسرطن لمتبقيات هرمون -17 بيتا استراديول ولمدة ستة اشهر على الصورة الدموية، حيث لم تظهر فروقات معنوية على خضاب الدم (11,5 و 11,69 غم / ديسيلتر)، العدد الكلي لخلايا الدم الحمراء (7,48 و 6,98 × 10 6 / مليمتر3)، الخلايا القعدة (3,40 و 3,54 %) و الخلايا الوحيدة (8,68 و 8,09%) و الخلايا الوحيدة (8,68 و 8,09 %) لكلا مجموعتي التجربة المعاملة و غير المعاملة على التوالي. وأظهرت قلة معنوية (P<0.05) على حجم الخلايا المرصوصة (37,40 و 31,28%)، الخلايا اللمفاوية (49 و 46,03 %)، وحساب خلايا الدم البيض (7,05 و 9,43× 10 9 / لتر) و الخلايا العدلة (35,52 و 37,35 %)، بينما أظهرت الخلايا الحمضية زيادة معنوية (P<0.05) في مجموعتي التجربة المعاملة و غير المعاملة (3,40 و 4,99 %) على التوالي. وأظهرت التأثيرات الخلوية (معامل الانقسام الخلوي) زيادة معنوية (P<0.05) في الفئران المعاملة عند مقارنتها بالمجموعة الغير معاملة. أوضحت التغيرات المرضية النسيجية في مبيض فئران المجموعة المعاملة بهرمون -17 بيتا استراديول 420 جزء بالبليون تطور للجريبات الغير الناضج مع ملاحظة الجريبات الاولية بينما لم يلاحظ وجود البيضة في الجريبات الثانوية ولا الجريبات القفاعية عند مقارنتها بمجموعة السيطرة الغير معاملة. فيما كانت التغيرات المرضية النسيجية لرحم فئران المجموعة المعاملة بملاحظة توسع في بطانة الغدة الرحمية مع فرط تنسج للخلايا الظهارية المبطنة للرحم وفرط خلوي مدمج في السدى. بينما أظهرت التغيرات العيانية للغدة اللبنية ظهور ورم غدي سرطاني مع كبر غير منتظم الشكل للغدة اللبنية لفئران المجموعة المعاملة بهرمون -17 بيتا استراديول فيما كانت التغيرات المرضية النسيجية في نفس المجموعة ظهور فرط تصبغ متعدد الاشكال للخلايا الخبيثة التي تأخذ تراكيب غدية.