

## **EFFECT OF OVER DOSE SYNTHETIC ESTRADIAL 17- $\beta$ HORMONE ON SOME PERIPHERAL BLOOD PARAMETERS AND BONE MARROW STEM CELLS IN ADULT FEMALE RATS**

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### **ABSTRACT**

This research was conducted on Animal Production Department's laboratories / University of Duhok, from the beginning of July to the end of October 2006, by using (36) female rats to investigate the effect of over dose of gonad hormone (estradiol 17- $\beta$ ) with dose of 0.5 mg injected intraperitoneally twice a day for 1 week. The rats were divided into three groups (12 rats for each) the first group was the control and the second injected with estradiol, while the third group was as the second one but the measurement of studied parameters were taken after 7 days after hormone injection. Some hematological parameters from blood samples and stem's cells of bone marrow were studied at 12 weeks of rats age. The results showed no significant differences effect in the (RBC) count , (Hb), Basophiles percent and Band Basophiles among the three experimental groups, while significant ( $P \leq 0.05$ ) depression in PCV among the control and the third group , second group, respectively. Significant ( $P \leq 0.05$ ) increase were observed in WBC between the control and the second group , third group and significance ( $P \leq 0.05$ ) increase in the Neutrophiles and Monocytes between the control and the third group and the second and the third group, respectively, as compared with significant ( $P \leq 0.05$ ) decrease in the Lymphocytes between the control and the third group and the two treated groups. However, significant difference ( $P \leq 0.05$ ) decrease in Early erythrocytes were noticed between control and the second group and significant ( $P \leq 0.05$ ) increase between the control and the third group and the two treated groups respectively, while significant ( $P \leq 0.05$ ) increase were noticed in Intermediate erythrocytes and Late erythrocytes and Band neutrophiles between the three groups respectively. Also significant ( $P \leq 0.05$ ) increase in Eosinophiles % and Band eosinophiles were noticed between the control and the second group , third group, and significant ( $P \leq 0.05$ ) increase in Myelocytes number and Megacaryocytes were noticed between the second and the third group only. We concluded that the estradiol 17-  $\beta$  treatment neither suppress erthropoiesis nor increase the immune system cells, but it caused a lymphoma in the peripheral blood.

### **INTRODUCTION**

Estrogen is one of the steroidal reproductive hormones, of all steroidal hormones having a tetra cyclic configuration (cholesterol is a common precursor of all steroidal hormones. Estradiol is a naturally occurring steroidal estrogen. The most active endogenous estrogen is estradiol possesses the pharmacologic activities of the estrogen class. Estrogens are necessary for the normal growth and development of the females and contribute to the

development and maintenance of secondary female sex characteristics. Estrogen causes increasing cell height and secretions of the cervical mucosa, and increased uterine tone (Susan *et al.*, 2003).

Estrogen have an effect on skeletal system, they increase calcium deposition and accelerate epiphysis closure and increase bone formation, estrogen has a slight anabolic effects and can increase sodium and water retention, estrogen also have an effect on the releasing of gonadotropins from the pituitary gland, this can cause inhibition of ovulation and inhibition of androgen secretion (Susan *et al.*, 2003). This hormone secreted by the ovary, testes, placenta and adrenal cortex, have a basic nucleus called cyclopentane perhydrophenantherene, in blood plasma, steroid hormones are mostly bound to albumin, plasma protein with low affinity and high capacity for steroids. Another portion of the steroid hormones is bound to one or more specific proteins with high affinity. Several substances of estrogenic activity are found in both the animal and plant kingdom.

Estradiol 17- $\beta$  is the biologically active estrogen produced by the ovary (Hafez and Hafez J, 2000). Estrogen's ability to influence osteoclastic activity has been well documented especially in post menopausal osteoporosis. High-dose of estrogen stimulates both new modularly bone formation in mouse long-bones and suppress hematopoiesis (Perry *et al.*, 2000).

In early results obtained in mice, an inability effect of physiological doses of the estrogen on several hematopoietic parameters including granulopoiesis and thrombocytopoiesis to replacement bone-marrow by new bone because estrogen induces osteosclerosis (Morse *et al.*, 2000). However, it has been shown that the effect of estrogen on murine hematopoiesis preceded those on bone formation, and providing evidence for a primary action of the hormone on the hematopoietic marrow (Perry *et al.*, 2000). Loss of gonad function causes metabolic changes that are associated with a reduction in bone mass caused by a yet undefined mechanism, this will cause increase in a deposits in the bone marrow associated with thinning of orbicular bone and leads to bone atrophy (Martin and Zissimos, 1991; Nuttall *et al.*, 1998; Cirotteau, 1999).

The aim of the study was to investigate the effect of over dose of gonad hormone (estradiol 17- $\beta$ ) on some peripheral blood parameters (blood pictures) and bone marrow stem cells in adult female rats.

## **MATERIALS AND METHODS**

This research was conducted in the Animal Production Department / University of Duhok, from the beginning of July to the end of October 2006, by using (36) female rats to investigate the effect of over dose of gonad hormone (estradiol 17- $\beta$ ) on some blood parameters and stem's cells. Rats were divided into three groups , 12 rats each.

1<sup>st</sup> group was the control, the 2<sup>nd</sup> group treated with estradiol 17- $\beta$  0.5 mg / animal

intraperitoneally twice daily for 1 week. Samples were collected at the end of treatment. 3<sup>rd</sup> group the same treatment as the 2<sup>nd</sup> group but the samples were collected 7 days after the end of treatment. Some hematological parameters from blood samples and stem's cells of bone

marrow were studied at 12 weeks of rats age. Blood samples were collected from anesthetized and slaughtered rats by heparinized tubes to measure RBC, WBC count, Hb %, PCV % and samples of bone marrow were taken from each rat by aspiration method, to measure Band neutrophiles, Band eosinophiles, Band basophiles, Myelocytes, Megakaryocytes cells and all stages of Erythropoiesis.

SAS Package (SAS,2002) was used to analyze the data statistically, using Analysis of Variance with Complete Randomized Design (CRD) , and New Duncan Multiple Range Test to test the significance of differences between treatment means.

## RESULTS AND DISCUSSION

The results that presented in Tables (1) declared that administration of estradiol 17- $\beta$  for 7 days had non-significant differences in the total number of red blood cells (RBC) counts in the peripheral blood, RBC counts were 7.05, 6.58 and  $6.76 \times 10^6/\text{mm}^3$  for the three groups respectively, and on blood hemoglobin (Hb) (16.05, 16.25 and 15.25 gm/dl ), also for Basophiles percent (0.25, 0.30 and 0.42 %) for the three experimental groups respectively, while significant ( $P \leq 0.05$ ) decrease in PCV were noticed between the control and the third groups (41.25 and 31.88 %) and the control and the second groups (41.25 and 34.50 %) respectively. Also significant ( $P \leq 0.05$ ) increase were observed in WBC count between the control and the second groups (5262.50 and 7044.17 / $\text{mm}^3$ ) and the control and the third group (5262.50 and 6410.67 / $\text{mm}^3$ ). The results presented in table (1) also showed significance ( $P \leq 0.05$ ) increase in the Neutrophiles and Monocytes number between the control and the third groups (23.67 and 32.88 % for Neutrophiles ) and (4.25 and 7.28 % for Monocytes ) and between the second and the third groups (24.96 and 32.43 % for Neutrophiles and 3.79 and 7.28 % for Monocytes) respectively, as compared with significant ( $P \leq 0.05$ ) decrease of the lymphocytes between the control and the third groups (71.41 and 57.15 %) and the two treated groups (68.54 and 57.15 %), respectively.

The results also revealed that administration of estradiol 17- $\beta$  for 7 days had a non-significant differences effects in the Band Basophiles (1.70, 1.61 and 1.94 %) for the three groups respectively, while significant ( $P \leq 0.05$ ) increasing were noticed in Intermediate erythrocytes, Late erythrocytes and Band neutrophiles for the three groups (13.08, 23.91 and 43.82 % for Intermediate erythrocytes and 17.49, 21.08 and 24.83 % for Late erythrocytes and 1.99, 13.38 and 19.58 % for Band neutrophiles), respectively.

Significant decrease ( $P \leq 0.05$ ) again was noticed in early erythrocytes between control and the second group (7.42 and 2.48 %) and significant increase ( $P \leq 0.05$ ) between the control and the third groups (7.42 and 12.57 %) and the two treated groups (2.48 and 12.57 %) ,respectively.

The results also showed significant ( $P \leq 0.05$ ) increase in Band eosinophiles between the control and the second groups (6.03 and 11.38 %) and the control and the third groups (6.03 and 12.88 %) respectively. Meanwhile significant ( $P \leq 0.05$ ) increase in Myelocyte number and Megakaryocytes were noticed between the second and the

third groups only (2.88 and 4.98 % for Myelocytes and 2.19 and 1.20 % for Megacaryocytes), respectively.

We concluded that the estradiol 17- $\beta$  neither suppress erthropoiesis nor increase the immune system cells. Estradiol 17- $\beta$  cause lymphoma in the peripheral blood.

Table (1) : Effect of estradiol 17- $\beta$  on some blood pictures and bone marrow stem cells in adult female rats.

Studied parameters	Group A (Control)	Group B (Treatment)	Group C After treatment)
RBC * 10 <sup>6</sup> /mm <sup>3</sup>	7.05 $\pm$ 0.16 a	6.58 $\pm$ 0.19 a	6.76 $\pm$ 0.29 a
Hb gm/dl	16.05 $\pm$ 0.22 a	16.25 $\pm$ 0.30 a	15.25 $\pm$ 0.64 a
PCV %	41.25 $\pm$ 0.50 a	34.50 $\pm$ 1.00 b	31.88 $\pm$ 1.40 b
WBC /mm <sup>3</sup>	5262.50 $\pm$ 130.1 b	7044.17 $\pm$ 347.6 a	6410.67 $\pm$ 479.6 a
Neutrophiles %	23.67 $\pm$ 2.11 b	24.96 $\pm$ 1.30 b	32.43 $\pm$ 1.68 a
Eosinophiles %	0.42 $\pm$ 0.24 b	2.42 $\pm$ 0.47 a	2.71 $\pm$ 0.47 a
Basophiles %	0.25 $\pm$ 0.17 a	0.29 $\pm$ 0.08 a	0.43 $\pm$ 0.14 a
Monocytes %	4.25 $\pm$ 0.70 b	3.79 $\pm$ 0.66 b	7.28 $\pm$ 0.72 a
Lymphocytes	71.41 $\pm$ 2.56 a	68.54 $\pm$ 2.83 a	57.15 $\pm$ 3.05 b
Early erythrocytes %	7.42 $\pm$ 0.92 b	2.48 $\pm$ 0.16 c	12.57 $\pm$ 0.11 a
Intermediate erythrocytes %	13.08 $\pm$ 0.88 c	23.91 $\pm$ 0.57 b	43.82 $\pm$ 1.98 a
Late erythrocytes %	17.49 $\pm$ 1.14 c	21.08 $\pm$ 0.31 b	24.83 $\pm$ 0.14 a
Myelocytes %	4.44 $\pm$ 0.78 ab	2.88 $\pm$ 0.12 b	4.98 $\pm$ 0.02 a
Band neutrophiles %	1.99 $\pm$ 0.07 c	13.38 $\pm$ 1.24 b	19.58 $\pm$ 0.69 a
Band eosinophiles %	6.03 $\pm$ 0.49 b	11.38 $\pm$ 1.16 a	12.88 $\pm$ 0.20 a
Band basophiles %	1.70 $\pm$ 0.05 a	1.61 $\pm$ 0.10 a	1.94 $\pm$ 0.07 a
Megacaryocytes %	1.61 $\pm$ 0.26 ab	2.19 $\pm$ 0.12 a	1.20 $\pm$ 0.04 b

\*Means with different letters were significantly different ( $p \leq 0.05$ ) according to New Duncan Multiple Range Test.

The gonad hormones deficiency change cells differentiation, these changes result from reduction in osteoblastic activity associated with an increase in adiposities in the bone marrow. Control data were almost parallel with previous reports of Denlaney (1996) who reported that decline of mesenchymal stem cells pool depleted animal lead to decrease of bone formation, and estrogen effects various cellular activities such as protein synthesis, ALK-P activity.

The non-significant differences which obtained of red blood cells count and hemoglobin, because of estradiol 17- $\beta$  as steroidal hormone secreted by the ovaries which enhance lipid metabolism and increase sedimentation rate of RBC and lower the count of RBC cells (Khan and Zafar, 2005). My result which in accordance to the finding of Gilbert (1962) and Nirmalan and Robinson (1972), who found a decrease in total RBC count in broiler chicken, non-significant difference in hemoglobin concentration and non-significance decrees in RBC number. However, my results not agreed with the results of Gilberts (1963) and Nirmalan and Robinson (1972) and Khan and Zafar (2005). Estradiol enhance lipid metabolism, increase sedimentation rate of RBC and lowered the count of RBC cells and inducing hyperlipemia and hemodilution. The unchange in RBC count and

hemoglobin concentration with the of high-significant increase in erythrocytes in the bone marrow probably may probably due to insufficient amount of vitamin B12 and folic acid to make final maturation of erythrocytes.

Conversely, the amount of PCV were reduced significantly during treatment and got more reduction after 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 erythropoiesis proliferation and differentiation arrest, resulted in increasing production of RBC (partially immature) , these initial results were almost comparable to that reported by Luger *et al.* (2003). Although my results marked no statistical differences in RBC count and hemoglobin concentration or hematocrit indices with references to hormones. These results were similar to that of Gilbert (1963 ) with continued estrogen administration in chickens, erythropoiesis is not depressed.

The increasing of WBC count after treatment may be due to the temporary increasing of estrogen. The result was similar to that of Ugochukwu *et al.* (2008). 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 (Nilsson , 2007).

The non-significant increase in basophiles during treatment and after treatment occurred (Northern *et al.*, 1994 ; Aspeloff *et al.*, 2000 ; Fass *et al.*, 2000 ; Bouman *et al.*, 2001 ; Nieuwenhoven *et al.*, 2002) 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 feed back 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 Yada *et al.* (2006) and Nilsson (2007). Such decrease and then return to the normal range after administration leads to the suggestion that estradiol inhibit the monocytes chemoattractant protein-1-induced monocytes migration through non-genomic estrogen receptor alpha. This may explain one of the anti-atherosclerotic effect of estradiol on vasculature. The results of this study were similar to that of Mediana *et al.* (1993) who reported that lymphopoiesis in murine bone marrow was suppressed during pregnancy (high level of progesterone ) , also they showed that estrogen selectively suppressed lymphocyte precursors when given to normal female rat.

It was noted that estradiol hormone differentially affect lymphopoiesis or myelopoiesis, it was demonstrated in the mouse model that shortly after ovariectomized surgery that lymphocytes were increase and the Myelocytes were decreased or did not changed appreciably (Smithson *et al.*, 1995), the study showed that deficiency of sex hormone increase lymphopoiesis and bone loss.

تأثير الجرعات العالية من هرمون الأسترايول 17- بيتا المصنع في بعض قياسات الدم والخلايا الجذعية  
لنخاع العظم في إناث الجرذان البالغة  
أراز جعفر محمد علي بيدايوي  
قسم الإنتاج الحيواني / كلية الزراعة / جامعة دهوك - العراق

الخلاصة

أجري هذا البحث في مختبرات قسم الإنتاج الحيواني / جامعة دهوك في بداية تموز وحتى نهاية تشرين الأول من سنة 2006 ، باستخدام (36) أنثى من إناث الجرذان للكشف عن تأثير الجرعات العالية للهرمون الجنسي ( استرايول 17- بيتا ) بجرعة 0.5 ملغم مرتين في اليوم عن طريق الحقن تحت الجلد لمنطقة البطن . قسمت إناث الجرذان إلى 3 مجاميع ، الأولى مجموعة السيطرة ، و الثانية حققت بهرمون ( estradiol 17-β ) بجرعة 0.5 ملغم/حيوان مرتين يوميا في الخلب لمدة أسبوع ، وسحبت النماذج الدموية مباشرة عند نهاية المعاملة أما المجموعة الثالثة فكانت نفس معاملة المجموعة الثانية ولكن تم سحب النماذج بعد أسبوع من نهاية المعاملة تم خلالها دراسة بعض الصفات الدموية وخلايا النخاع عند 12 أسبوع من عمر إناث الجرذان . لم تظهر النتائج فروقات معنوية لتأثير الهرمون في معدل كريات الدم الحمراء و هيموكلوبين الدم ونسبة الخلايا القاعدية والخلايا الحزمية القاعدية بين المجاميع التجريبية الثلاثة ، في حين وجد انخفاض معنوي ( $\geq 0.05$ ) في معدل الخلايا المتراصة بين مجموعتي معاملة السيطرة و المعاملة الثالثة من جهة وبين معاملة السيطرة ومجموعة المعاملة الثانية على التوالي. كما لوحظ أيضاً زيادة معنوية ( $\geq 0.05$ ) في معدل كريات الدم البيض بين مجموعة معاملة السيطرة و مجموعة المعاملة الثانية وبين مجموعة معاملة السيطرة ومجموعة المعاملة الثالثة على التوالي. في حين لوحظ زيادة معنوية ( $\geq 0.05$ ) في أعداد النيوتروفيل والمونوسايت بين مجموعة معاملة السيطرة ومجموعة المعاملة الثالثة من جهة وبين مجموعة المعاملة الثانية ومجموعة المعاملة الثالثة من جهة أخرى مقارنة بالانخفاض المعنوي ( $\geq 0.05$ ) للخلايا اللمفية بين مجموعة معاملة السيطرة ومجموعة المعاملة الثالثة وبين المجموعتان اللتان عوملتا بالهرمون . على كل حال ، وجد انخفاض معنوي ( $\geq 0.05$ ) في عدد الخلايا البيضاء المبكرة بين مجموعة معاملة السيطرة ومجموعة المعاملة الثانية وزيادة معنوية ( $\geq 0.05$ ) بين مجموعة معاملة السيطرة ومجموعة المعاملة الثالثة وبين المجموعتان اللتان عوملتا بالهرمون . على كل حال ، وجد انخفاض معنوي ( $\geq 0.05$ ) في عدد الخلايا البيضاء المبكرة بين مجموعة معاملة السيطرة ومجموعة المعاملة الثانية وزيادة معنوية ( $\geq 0.05$ ) في أعداد المايكروسايت والميكاكاربوسايت بين المجموعة الثانية والمجموعة الثالثة فقط . نستنتج من ذلك بأن حقن هرمون الأسترايول 17- بيتا لا يخفض من عملية تكوين الخلايا الحمراء في الدم ونخاع العظم أو يزيد من الخلايا المناعية ولكن حقنه يعمل على خفض الخلايا اللمفية في الدم مسبباً ورم ليفي.

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