

Preface

قال تعالى:

﴿ بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ ﴾

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنْسَانَ
مِنْ عَلَقٍ ﴿٢﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾ الَّذِي
عَلَّمَ بِالْقَلَمِ ﴿٤﴾ الْإِنْسَانَ مَا لَمْ
يَعْلَمْ ﴿٥﴾

صدق الله العظيم

سورة العلق (١-٥)

Dedication

To those who are giving me the love and support, my parents

**To those who are surrounding me by love and respect, my wives;
Madeha Ahmed and Hedia Zolain.**

To my lovely kids ; Dalia, Mohamod & Abdallaha

To my supervisor & colleagues, I dedicated this work.

Acknowledgement

My first and last thanks to ALLAH who gave me ability to carry out this work. My deep thanks and respect to my supervisors; Associated Prof. **Shams-Eldein Hassaballa** and prof **Shadia Abdoelatti Omer**, for their help, guidance and patience to bring out this work . My thanks to University of Nyala and Deutscher Akademischer Austausch Dienst (DAAD) for their financial support .My great thanks without limit to all my colleagues , Abdalla Ibnomer, Hedia Zolain, Abdelnasir Adam and Eltahier Shuiep.

Finally to all those who I didn't mention my deep respect and a lot of thanks.

Elkhier Tamour Ahmed Tamour

Abstract

This study was conducted to investigate the effect of injection of Estradiol Benzoate to intact and castrated male rabbits on some blood constituents and prostate tissue. A total of Ninety mature male rabbits (54 intact and 36 castrated) were used in this study. The rabbits were randomly divided into three groups and each group was further divided into four sub-groups. The rabbits of each groups were injected estradiol benzoate dosed at 0, 40, 80 and 120 $\mu\text{gm}/\text{rabbit}$, through intramuscular (IM) route, on each alternative day over a period of 30 days, whereas group three (18 intact rabbits) were offered an oral dosage of 1mg from prolactin inhibitor (Bromocriptine Mesilate)/ rabbit in addition to estradiol. Serum LH, FSH, and Testosterone levels were performed by immunoenzymometric assay , total-protein, Albumin, glucose , urea , creatinine, lipids profiles, AST, ALT and ALP enzymes, were measured in serum samples. Automate chemistry analyser (MINDARY) and reagent Biosystem® Spain made were used for analysis .

Prostate tissue samples were taken from each sub-groups, tissue processing method was made by automatic tissue processor and then embedded in paraffin wax. Sections of 5 μ thick were cut by a rotary microtome, stained in Mayer's haematoxylin and Eosin H&E (Bancroft et al., 1996). All sections were examined under the light microscope (Olympus) to describe the microscopic changes and imaged by using digital camera. The statistical analyses were done using the SPSS statistical program, version 20 for Windows (IBM SPSS Statistics 20 IL, USA), and the results were expressed in form of mean \pm standard deviation. The results showed that, the mean serum levels of LH and FSH were not affected by injection of estradiol benzoate in all intact and castrated rabbits sub-groups ($P > 0.05$), while the mean testosterone concentration levels were showed insignificant increase in both intact and castrated male rabbits, except the intact male rabbit sub-group that

received estradiol benzoate at 120 µg/rabbit ($P < 0.05$). However, the concentration levels of total protein, glucose, albumin and calcium were not affected by the injection of different doses of estradiol benzoate. Moreover, the study revealed that, the injection of estradiol benzoate at a dose of 40 µg and 120 µg IM to intact male rabbits induced a significant ($P < 0.05$) decrease in the creatinine level and increase in urea concentration in castrated rabbits. However, the result of the present study showed that, the injection of estradiol benzoate at doses of 40, 80 and 120 µgm/rabbit intramuscularly were not induced any significant ($P > 0.05$) changes on lipids profile of intact and castrated male rabbits, while HDL was significantly ($P < 0.05$) increased at a dose 40 µg in intact male rabbits. However, liver enzymes AST, ALT and ALP did not affected ($P < 0.05$) by injection of estradiol benzoate to intact rabbits, whereas, AST and ALP were significantly ($P < 0.05$) decreased in castrated male rabbits as compare to the intact male rabbits. The correlation between estradiol benzoate doses and blood constituents had showed that, a significant positive correlation in ALT, testosterone and significant negative correlations in glucose, triglyceride, LDL and LH in intact male rabbits, whereas, a significant positive correlation in glucose, urea, and a significant negative correlations in creatinine, triglyceride, AST, and ALP. And in castrated male rabbits were detected. Furthermore, the effects of estradiol benzoate in prostate tissues were ranged from hyperplasia with dysplasia or dysplasia only in intact male rabbits; hyperplasia was represented by papillary projection in castrated male rabbits, however, administration of estradiol benzoate to intact male rabbits in concomitance with prolactin inhibitor (Bromocriptin mesylate) induced mild hyperplasia in intact male rabbits.

It is concluded that injection of estradiol benzoate to male rabbits induced many effects represented in LH, FSH, testosterone, total-protein, Albumin, glucose, urea, creatinine, lipids profiles, liver enzymes, there is however, strong evidence shows

that excessive or untimely exposure to estrogens can facilitate development of prostatic changes, disorders and even malignancies.

مستخلص الأطروحة

أجريت هذه الدراسة لتقصي التغيرات في بعض مكونات الدم ونسج غدة البروستات نتيجة للحقن بالاستراديول بنزويت لذكور الارانب غير المخصيه والمخصيه. عدد تسعون ذكر بالغ من الارانب (54 غير مخصيه و 36 مخصيه) تم استخدامها في هذه الدراسة. وزعت الارانب عشوائيا الي ثلاث مجموعات وكل مجموعة قسمت لاحقا الي اربعة مجموعات فرعية. الارانب في كل مجموعة حققت استراديول بنزويت بجرعات 0، 40، 80 و 120 ميكروجرام/ارنب عن طريق العضل يوم بعد يوم لفترة 30 يوم، بينما المجموعة الثالثة (احتوت علي 18 ارنب) اعطيت جرعات فموية بمقدار 1 ملجم من مثبط البرولاكتين (بروم كربتئين ميثاليت)/ ارنب بالاضافة للاستراديول. مستوى الهرمونات المصفر، المحفز لنمو الجريبات ، و التستوستيرون تم قياسها باستخدام طريقة القياس الانزيمي المناعي، البروتين الكلي ، الألبومين، الجلوكوز ، اليوريا، الكرياتين، الدهون، الانزيمات إسبارتات ترانساميناز ، الأنين ترانساميناز ، و الفوسفاتاز القلوي تم قياسها باستخدام جهاز التحليل الكيميائي الاتوماتيكي (MINDARY) ووسيط التفاعل Biosystem® اسباني الصنع. تم اخذ عينات لنسج البروستات من كل المجموعات الفرعية، تمت معالجة انسجة البروستات بواسطة جهاز معالجة الانسجة الاتوماتيكي ومن ثم طمرت في شمع البرافين. قطعة سمكها 5 ميكرون تم قطعها باستخدام جهاز التقطيع الدوار ، وتم صبغها بصبغة مايير H&E حسب وصف (Bancroft et al., 1996). كل المقاطع النسيجية تم فحصها تحت المجهر الضوئي (Olympus) لوصف التغيرات المجهرية وتصويرها بالكاميرة الرقمية . التحليل الاحصائي تم باستخدام البرنامج الاحصائي SPSS طراز 20 لنظام التشغيل ويندوز, (IBM SPSS Statistics 20 IL, USA) ، وتم التعبير عن النتائج في شكل المتوسط \pm الانحراف المعياري.

. أظهرت النتائج بان متوسط مستوي السيرم للهرمونات المصفر والمحفز لنمو الجريبات لم يتأثر بحقن الاستراديول بنزويت في كل المجموعات الفرعية للارانب غير المخصيه والمخصيه ($P>0.05$) ، بينما متوسط مستوي تركيز التستوستيرون اظهر ارتفاع غير معنوي في كل من الذكور غير المخصيه والمخصيه ماعدا في الذكور غير المخصيه للمجموعة الفرعية التي اعطيت استراديول بنزويت بجرعة 120 ميكروجرام /أرنب. بينما مستوي تركيز البروتين الكلي ، الالبومين، الجلوكوز ، والكالسيوم لم تتأثر بحقن الجرعات المختلفة للاستراديول بنزويت. وعلاوة على ذلك، كشفت الدراسة أن حقن الاستراديول بنزويت بجرعات 40 و 120ميكروجرام عن طريق العضل احدث نقصان معنوي ($P<0.05$) للكرياتين للذكور غير المخصيه ، وزيادة معنوية ($P<0.05$) لليوريا عند الذكور المخصيه. ومع ذلك، أظهرت نتيجة الدراسة الحالية أن حقن الاستراديول بنزويت بجرعات 40 و 80 و 120 ميكروجرام / أرنب بالعضل لم تحدث أي تغييرات معنوية كبيرة ($P>0.05$) على الدهون في كل من الذكور غير المخصيه والمخصيه، بينما البروتين الدهني عالي الكثافة ازداد معنويا ($P<0.05$) عند الجرعة 40 ميكروجرام في الذكور غير المخصيه. مع ذلك انزيمات الكبد إسبارتات ترانساميناز ، الأنين ترانساميناز ، و الفوسفاتاز القلوي لم تتأثر ($P>0.05$) بحقن الاستراديول بنزويت للذكور غير المخصيه. بينما إسبارتات ترانساميناز و الفوسفاتاز القلوي انخفضت معنويا ($P<0.05$) عند الذكور المخصيه بالمقارنة مع الذكور غير المخصيه .

الارتباط بين جرعات الاستراديول بنزوييت و مكونات الدم في ذكور الارانب غير المخصيه اظهر وجود ارتباط معنوي موجب في الأئين ترانساميناز و التستوستيرون . ووجود ارتباط معنوي سالب في الجلوكوز ، الجليسيريدات الثلاثية ، البروتين الدهني منخفض الكثافة و الهرمون المصفر. و في ذكور الارانب المخصيه اظهر وجود ارتباط معنوي موجب في الجلوكوز واليوريا ووجود ارتباط معنوي سالب في الكرياتينين ، الجليسيريدات الثلاثية ، إسبارتات ترانساميناز و الفوسفاتاز القلوي . علاوة على ذلك، تأثيرات الحقن بالاستراديول بنزوييت علي نسيج البروستات ترواح من فرط في النسيج وتغير في شكل الخلايا او تغير شكل الخلايا فقط عند ذكور الارانب غير المخصيه ، فرض في النسيج متمثلا ببروزات حلليميه عند ذكور الارانب المخصيه. وعلی الجانب الآخر حقن الاستراديول بالتزامن مع مثبط البرولاكتين (البروكربيتين ميثاليت) احدث فرض تنسج خفيف بنزوييت لذكور الارانب غير المخصيه. وخلصت الدراسة بان حقن الاستراديول بنزوييت لذكور الارانب له تأثيرات عديدة علي كل من الهرمونات المصفر ، محفز الجريبات والتستوستيرون، البوتين الكلي ، الالبومين ، الجلوكوز، اليوريا ، الكرياتينين، الدهون، انزيمات الكبد، ولكن هناك أدلة قوية تبين أن التعرض المفرط أو غير الملائم للأستروجين يمكن أن يسهل تطور التغيرات في البروستات، والاضطرابات وحتى الأورام الخبيثة.

List of contents:

Items	Page
Preface	I
Dedication	II
Acknowledgement	III
Abstract	IV
Arabic abstract	VII
List of contents	IX
List of Table	XVII
List of figures	XVIII
List of abbreviations	XIX
Introduction	XXI
CHAPTER ONE	
1 literature review	2
1.1 Estrogens	2
1.2 Chemical structure of estrogens and estradiol benzoate	3
1.3 The Sources of estrogen in male	4
1.4 Estrogen concentrations in semen and fluids of the male reproductive tract	5
1.5 Estrogen Receptors in the Male Reproductive Tract	7
1.6 Xenoestrogens	7
1.6.1 Diethylstilbestrol (DES)	7

1.6.2	Bisphenol A (BPA)	9
1.6.3	Alkylphenols	9
1.7	Differences between the action of natural and synthetic steroid hormones	9
1.8	The effects of estrogen on serum biochemical parameters	10
1.8.1	Glucose concentration	10
1.8.2	Total protein	11
1.8.3	Albumin	12
1.8.4	Calcium	12
1.8.5	Creatinine	13
1.8.6	Urea	14
1.8.7	Cholesterol, High Density Lipoprotein (HDL) Low Density Lipoprotein (LDL) , very Low Density Lipoprotein (VLDL) and Triglycerides	15
1.8.8	Aspartate Aminotransferase (AST) ,Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP)	17
1.8.9	Luteinizing hormone (LH) and follicle-stimulating hormone (FSH)	17
1.8.10	Testosterone	18
1.9	The Accessory sex glands	18
1.9.1	Functions of accessory sex glands	19
1.9.2	Species variation in accessory sex glands	19

1.9.3	Prostate gland	20
1.9.3.1	General function of prostate gland	20
1.9.3.2	The arrangement of the prostatic lobes	20
1.9.3.3	The prostate gland of male rabbits	21
1.10	The effect of hormones on prostate gland	21
1.10.1	Estrogen	21
1.10.2	Testosterone	21
1.10.3	Prolactin	22
1.11	Role of prolactin in mediation of estrogen effect on prostate	23
1.12	Orchiectomy(surgical castration)	24
CHAPTER TWO		
2	Materials and Methods	26
2.1	Experimental animals	26
2.2	Experiment design	26
2.2.1	Experiment I	26
2.2.2	Experiment II	27
2.2.3	Experiment III	27
2.3	Estrogen administration protocols	28
2.3.1	Calculation of doses	28
2.3.2	Route of administration	28
2.4	Inhibition of Prolactin secretion	28
2.5	The castration process	28

2.6	Collection of samples	29
2.6.1	Blood collection	29
2.6.2	Prostate tissue collection	29
2.7	Analysis of samples	29

2.7.1	Serum analysis	29
2.7.1.1	Glucose	30
2.7.1.1.1	Principle of the method	29
2.7.1.1.2	The components of reagent	29
2.7.1.2	Total protein	30
2.7.1. 2.1	Principle of the method	30
2.7.1.2.2	The composition of reagents	31
2.7.1.3	Albumin	31
2.7.1.3.1	Principle of the method	31
2.7.1.3.2	The composition of reagents	31
2.7.1.4	Calcium	31
2.7.1.4.1	Principle of the method	32
2.7.1.4.2	The composition of reagents	32
2.7.1.5	Creatinine	32
2.7.1.5.1	Principle of the method	32
2.7.1.5.1	The composition of reagents	32
2.7.1.6	Urea	33

2.7.1.6.1	Principle of the method	33
2.7.1.6.2	The composition of reagents	33
2.7.1.7	Cholesterol	33
2.7.1.7.1	Principle of the method	34
2.7.1.7.2	The composition of reagents	34

2.7.1.8	High Density Lipoprotein (HDL)	34
2.7.1.8.1	Principle of the method	34
2.7.1.8.2	The composition of reagents	35
2.7.1.8.3	Additional reagents	35
2.7.1.9	Precipitation of (VLDL) (LDL) in the samples	35
2.7.1.10	Low Density Lipoprotein (LDL)	35
2.7.1.10.1	Principle of the method	36
2.7.1.10.2	The composition of precipitating reagents	36
2.7.1.10.3	Additional reagents	36
2.7.1.10.4	Precipitation procedure	36
2.7.1.11	Triglycerides	37
2.7.1.11.1	Principle of the method	36
2.7.1.11.2	The composition of reagents	37
2.7.1.12	Enzymes	37
2.7.1.12.1	Aspartate Aminotransferase (AST)	37
2.7.1.12.1.1	Principle of the method	38

2.7.1.12.1.2	The composition of reagents	38
2.7.1.12. 2	Alanine Aminotransferase (ALT)	38
2.7.1.12. 2.1	Principle of the method	38
2.7.1.12. 2.2	The composition of reagents	39
2.7.1.12.3	Alkaline phosphatase (ALP)	39
2.7.1.12.3.1	Principle of the method	39
2.7.1.12.3.2	The composition of reagents	39
2.7.1.13	Hormones FSH, LH, and Testosterone	40

2.7.2	Tissues analysis	40
2.7.2.1	Prostate histological change study	40
2.7.2.1.1	Fixation	40
2.7.2.1.2	Tissue processing	40
2.7.2.1.3	Microtomy	41
2.7.2.1.4	Staining with H&E	41
2.7.2.1.5	Microscopic examination	42
2.8	Statistical analysis	42

CHAPTER THREE

3	The results	44
3.1	The effects of Estradiol Benzoate on blood constituents of intact males rabbits	44
3.1.1	Concentration of Glucose, T-protein, Albumin,	44

	Globulin, Calcium, Urea, and Creatinine	
3.1.2	Lipids profile	46
3.1.3	AST, ALT and ALP Enzymes	47
3.1.4	FSH, LH and Testosterone hormones	48
3.2	The effects of Estradiol Benzoate on blood constituents of castrated males rabbits	49
3.2.1	Concentration of Glucose, T-protein, Albumin, Globulin, Calcium, Urea, and Creatinine	49
3.2.2	Lipids profile	51
3.2.3	AST, ALT and ALP Enzymes	52
3.2.4	FSH, LH and Testosterone hormones	53
4	The correlation between estradiol benzoate doses and blood biochemical parameters	54
4.1	Intact male rabbits	54
4.2	Castrated male rabbits	56
5	The effect of Estradiol on prostate glands tissue	58
5.1	Intact male rabbits	58
5.2	Castrated males rabbits	60
5.3	The effect of Estradiol benzoate on prostate tissue of intact males rabbits in presence of Prolactin inhibitor	62
CHAPTER FOUR		
4	Discussion	65
5	Conclusion	72

6	Recommendations	72
7	References	74
8	Appendix	98
9	The published papers	100

List of tables:

Table .NO	Items	Page
1	The effect of estradiol benzoate on concentration of Glucose, T-protein, Albumin, Globulin, Calcium, Urea, and Creatinine of intact male rabbits	45
2	The effects of estradiol benzoate on Lipids profile of intact male rabbits	46
3	The effects of estradiol benzoate on AST, ALT and ALP Enzymes of intact male rabbits	47
4	The effects of Estradiol benzoate on FSH, LH and testosterone hormones of intact male rabbits	48
5	The effect of estradiol benzoate on concentration of Glucose, T-protein, Albumin, Globulin, Calcium, Urea, and Creatinine of castrated male rabbits	50
6	The effects of estradiol benzoate on Lipids profile of castrated male rabbits	51
7	The effects of estradiol benzoate on AST, ALT and ALP Enzymes of castrated male rabbits	52
8	The effects of Estradiol benzoate on FSH, LH and testosterone hormones of castrated male rabbits	53

9	Correlation between estradiol benzoate doses and biochemical parameters of intact male rabbits	55
10	Correlation between estradiol benzoate doses and biochemical parameters of castrated male rabbits	57

List of figures

Figure .NO	Items	Page
1	The effect of Estradiol on prostate glands tissue of intact male rabbits	59
2	The effect of Estradiol on prostate glands tissue of castrated male rabbits	61
3	The effect of Estradiol benzoate on prostate tissue of intact males rabbits in presence of Prolactin inhibitor	63

List of Abbreviations:

Abbreviation	Stand for
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ALP	Alkaline phosphatase
BPA	Bisphenol A
BPH	Benign prostatic hyperplasia
C°	Degree centigrade
cm	Centimeter
DES	Diethylstilbestrol
DHT	Dihydro testosterone
dL	Deciliter
E2	Estrogen
EDC	Endocrine disrupting compound
ERs	Estrogen receptors
ER α	Estrogen receptors alpha
EU	European union
FSH	Follicle stimulating hormone
G	Gram
HDL	High density lipoprotein
H&E	Hematoxiline and Euosin
IFCC	International Federation of Clinical Chemistry
IM	Intramuscular
Kg	Kilogram
L	Liter

LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LH	Luteinizing hormone
mg	Milligram
ml	Milliliter
Mmol	Millimol
MDH	Malate dehydrogenase
Ng	Nano gram
Nm	Nanometer
nmol	Nano mol
NS	Normal saline
O-CPC	O-Cresolphthalein complexon
Pg	Pico gram
PIF	Prolactin inhibitory factor
PRL	Prolactin
PTH	Parathyroid hormone
RBCs	Red blood corpuscular
r.p.m	Round per minutes
SHBG	Sex hormone binding globulin
SPSS	Statistical package for social science
U	Unit
UGS	Urogenital sinus
VLDL	Very low density lipoprotein
μ	Micro

Introduction

Estrogens are steroid hormones include estrone, estradiol, and estriol which animal body is produce them naturally (Moskowitz, 2006), and they are regarded as the main reproductive hormones that affects growth, development, maturation and functioning of reproductive tract as well as the sexual differentiation and the behavior (Balthazart *et al.*, 2009). Estrogen was historically believed to be a female hormone, but there is growing evidence of a biological role of this steroid in the male reproduction (Tohidi, 2010). The source of estrogen in males may be germ cells (Carreau *et al.*, 2003), or liver, adrenal glands, adipose tissue, and testes (Tohidi, 2010). Beside indigenous estrogens, there were exogenous estrogens called Xenoestrogens which have been defined by The United States Environmental Protection Agency as “an exogenous agent” that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood borne hormones that are present in the body and which are responsible for homeostasis, reproduction and developmental process (Evanthia *et al.*, 2009). There were differences between the natural and synthetic steroids in their effects on body due to the difference in affinity for binding to globulin , biological activity in plasma, metabolism, and half-life (Anna-Maria and Niels,1999). The exogenous estrogenic compounds were used for anabolic purposes in food animals to increase the secretory characteristics of growth hormone (Misztal *et al.*, 2007 and Colak *et*

al., 2011) for weight gain purposes. Other exposure sources to these compounds may occur through industry , agriculture, and some of these compounds are used in food production and food packaging , diet (e.g. pesticide residues on fruit and vegetables, food contaminated by compounds found in can lining and plastic wrapping etc.), in addition to phytoestrogens, which are found in rich amount in certain plants. Although the endogenous estrogen have physiological roles in males (Nilsson *et al .*, 2001; O'Donnell *et al .*, 2001; Hess and Carnes, 2004 and Carreau *et al .*, 2008 and Tohidi, 2010), the exposure to exogenous estrogen can cause adverts effects such as erectile dysfunction and impotence (Adaikan and Srilatha, 2003), infertility (Rozati *et al .*, 2002), prostate cancer (Ritchie *et al.*, 2003) and testicular dysgenesis syndrome (TDS) (Sharpe, 2003).

The objectives of this study were:

1. To investigate the changes on concentrations of glucose, total protein, albumin, calcium, urea and creatinine, lipids profiles, AST, ALT and ALP enzymes of intact and castrated male rabbits due to exposure to different doses of estradiol benzoate.
2. To study the changes on serum concentration of reproductive hormones LH, FSH, testosterone.
3. To study the correlation between the estradiol doses and the changes in blood biochemical parameters.

4. To study the effect of estradiol benzoate on prostate tissue and the role of prolactin on estrogen action in prostate was also investigated.
5. To compare the effect of estrogen on prostate gland in presence and absence of testicular testosterone.