CHAPTER ONE

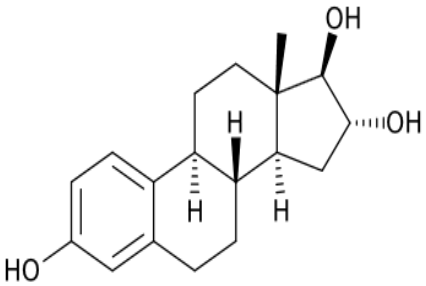
1- Literature Review

1.1-Estrogens:

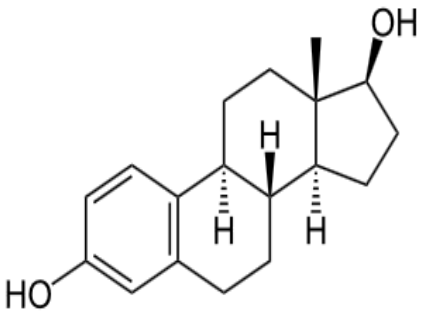
Estrogen is the major hormone that affects growth, development, maturation and functioning of reproductive tract as well as the sexual differentiation and behavior (Lien *et al* ., 1985 and Balthazart *et al*., 2009). An animal body naturally produces three main types of estrogen; these are estradiol-17-ß (E2), estrone and estriol (Moskowitz, 2006). Estrone and estriol were identified in the urine of pregnant women and this was followed by the identification of E2 in the follicular fluid of a sow by Edward Adelbert Doisy between 1929 and 1936 (Simoni *et al* .,2002). Estradiol-17 ß is the most physiologically active type of estrogen produced from testosterone and androstenedione, by aromatase which catalyzes an aromatic hydroxylation of the A ring of C19 androgens. The term estrogen is mainly used when referring to E2.

Although estrogens were historically believed to be a female hormone, there is growing evidence of their biological roles in male reproduction. (Todiodi, 2010).Estrogens play important roles in the development and maintenance of reproductive function and fertility (Nilsson et al ., 2001; O'Donnell *et al* ., 2001; Hess and Carnes, 2004 and Carreau *et al* ., 2008). Estrogens also have main role in pathological processes observed in tissues of the reproductive system (Prins and Korach, 2008 and Ellem and Risbridger, 2009).

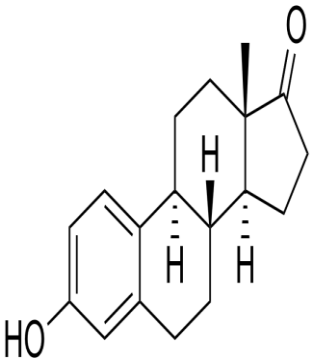
1.2-Chemical structure of estrogens and estradiol benzoate:



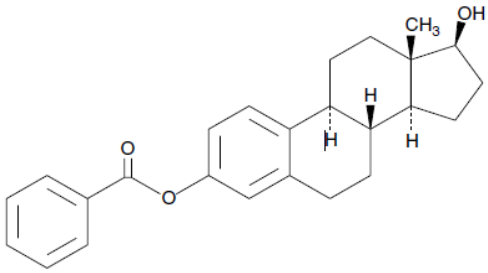
Estriol



Estradiol



Esteron



Estradiol Benzoate

1.3-The Sources of estrogen in the male:

Estrogen biosynthesis is catalyzed by a microsomal member of the cytochrome P450 super family, aromatase cytochrome P450 (Simpson *et al*., 1994). Aromatase expression was first described in germ cells of the adult mouse testis (Nitta *et al*., 1993).However, aromatase is expressed in the testis at all stages of development (O'Donnell *et al*., 2001), and estrogen production has been reported in fetal testis (Weniger *et al*., 1993), Leydig and Sertoli cells both contribute to estrogen production in the immature postnatal (Tsai-Morris *et al*., 1985; Dorrington and Khan, 1993), and adult testis (Carreau *et al*.,1999).

Early study by (van der Molen *et al* ., 1981) reported that Sertoli cells were the primary source of estrogen in the immature male. While (Rommerts and Brinkman, 1981, Rommerts *et al* ., 1982; Payne *et al*., 1987; Levallet *et al*., 1998, and Carreau *et al* ., 2003) were reported that Leydig cells in the adult testis express aromatase (P450arom) and actively synthesize estradiol at a rate much greater than that seen in the adult Sertoli cell. Estrogen may be produced by the liver, adrenal glands, adipose tissue, and testes (Todiodi, 2010). Currently, a growing body of evidence indicates that germ cells also synthesize estrogen, and possibly serve as the major source of this steroid in the male reproductive tract (Carreau *et al* ., 2003).

1.4-Estrogen concentrations in the males of animal:

Species	Source	Concentration	references
Horse	venous blood	450 ng/ml	(Setchell, and Cox 1982)
Boar	,,	1.09 nmol/L (total estrogens) 52.4 nmol/L (estrone-sulfate)	(Setchell *et al*., 1983)
Man	,,	926 pg/ml	(Adamopoulos *et al*., 1984)
Monkey	Testis	104-200 pg/ml	(Waites and Einer-Jensen, 1974)
Horse	Testis lymph	900 ng/ml (estrone-sulfate)	(Setchell and Cox, 1982)
Boar	,,	1.86 nmol/L (total estrogens) 705 nmol/L (estrone sulfate)	(Setchell *et al*., 1983)
Monkey	Rete testis fluid	14-195 pg/ml	(Waites and Einer-Jensen, 1974)
Rat	,,	249 pg/ml	(Free and Jaffe, 1979)
Bull	,,	11.5 pg/ml	(Ganjam and Amann, 1976)
Boar	,,	0.38 nmol/L (total estrogens) 8.60 nmol/L (estrone-	(Setchell *et al*., 1983)

		sulfate)	
Man	Semen	6.7-162 pg/ml	Bujan *et al.*, 1993; Luboshitzky *et al.*, 2002a,b; Naderi and Safarinejad, 2003)
Horse	,,	73- 144 pg/ml (estradiol) 385 pg/ml (conjugated estradiol) 739 pg/ml estrone 4116-9612 pg/ml (estrone-sulfate)	(Claus *et al.*, 1992; Lemazurier *et al.*, 2002)
Bull	,,	50-890 pg/ml	(Ganjam and Amann, 1976; Eiler and Graves, 1977)
Boar	,,	430 pg/ml (estradiol) 860 pg/ml (estrone)	(Claus *et al.*, 1985)

1.5-Estrogen Receptors in the Male Reproductive Tract

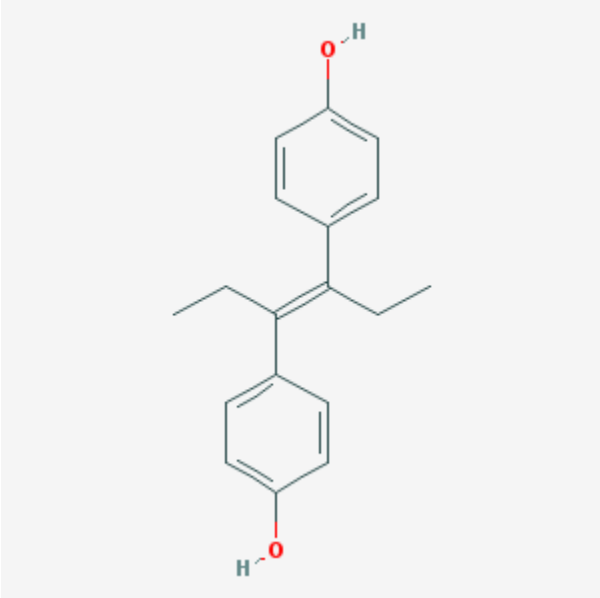
The actions of estrogen are mediated through estrogen receptors ERα and ERβ. Estrogen receptors are members of the steroid receptor super family of ligand-activated transcription factors (Evans, 1988). Estrogen receptors reside within the nucleus of target cells in an inactive state in the absence of ligand. Upon binding its ligand, 17β-estradiol (E2), the receptor undergoes an activating conformational change permitting it to interact with specific cofactors and bind DNA response elements within target gene promoters (Beekman *et al*.,1993., Horwitz *et al*., 1996).

1.6-Xenoestrogens:

Xenoestrogens means foreign estrogens and also called "environmental hormones" or endocrine disrupting compounds (EDC) and 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).

1.6.1-Diethylstilbestrol (DES):

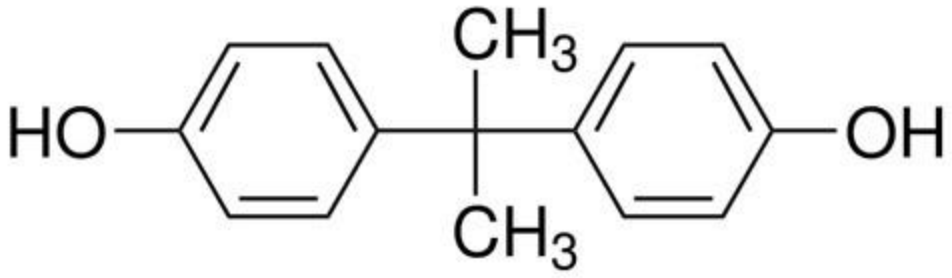
Diethylstilbestrol (DES) is synthetic potent non-steroidal estrogen used as a supplement in cattle and poultry feed and as a pharmaceutical (Rubin, 2007). Further studies have reported multiple adverse effects in males and females as a result of prenatal DES exposure.



Diethylstilbestrol (DES)

In males decreased fertility and anatomical malformations of reproductive organs such as cryptorchidism, epididymal cysts and prostatic squamous metaplasia were observed (Driscoll and Taylor, 1980; Marselos and Tomatis, 1992., Mittendorf,1995). In male mice exposed to DES during gestation, cryptorchidism, hypospadias, as well as underdeveloped epididymis, vasdeferens and seminal vesicles were observed (McLachlan *et al.*, 2001). Similarly, neonatal treatment of male rats with DES induced a wide range of reproductive abnormalities, including delay of testicular descent, retardation of pubertal spermatogenesis, reduction in testis weight, infertility, and gross morphological alterations in the rete testis, efferent ducts, epididymis and accessory sex glands (Atanassova *et al.*, 1999, 2000., Fisher *et al.*, 1999.,McKinnell *et al*, 2001., Williams *et al.*, 2001). Studies on transgenic mouse models within activated ERs suggest that DES elicits its toxic effects in the male reproductive tract through an ER**α**-mediated mechanism (Prins *et al*., 2001).

1.6.2-Bisphenol A (BPA)



Bisphenol A (BPA)Is one of the most important industrial chemicals, with worldwide production of over 500 000 tons per year. It is found mainly in plastic food containers, baby bottles, the resins lining food cans, dental sealants, cardboards, and as an additive in other plastics (Richter *et al*., 2007). BPA mimics estrogen action *in vivo* and in *vitro,* however it is also able to antagonize the estradiol activity, via modulation of selective estrogen receptor (Welshons *et al.,* 2006). It was demonstrated that rodents exposed to BPA during fetal and/or neonatal life had decreased weights of the epididymis and seminal vesicles, but increased weights of the prostate and preputial glands, decreased epithelial height in the efferent ducts and decreased levels of testicular testosterone (Akingbemi *et al*., 2004., Fisher *et al*., 1999., Vom Saal *et al*., 1998).

1.6.3-Alkylphenols:

Alkylphenols such as 4-nonylphenol and 4-*tert*-octylphenol, are used to manufacture the alkylphenol polyethoxylates, non-ionic surfactants used as detergents, plasticizers, emulsifiers and modifiers in paints, pesticides, textiles, and personal care products. Alkylphenols present in the environment, mainly in wastewater and rivers, derive from the release of un reacted alkylphenols during manufacturing as well as from degradation of the alkylphenol polyethoxylates in the environment (Blake *et al*., 2004; Staples *et al*., 2001).

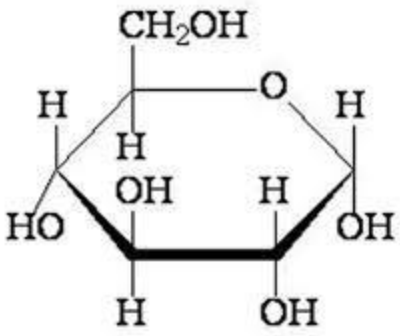
1.7-Differences between the action of natural and synthetic steroid hormones:

According to a review by (Anna-Maria and Niels ,1999) Many synthetic sex steroids have a low affinity for sex hormone binding globulin (SHBG) and are present in an unbound form and biologically active in plasma. In contrast, approximately 98–99% of the endogenous sex steroids are bound to SHBG in plasma. Therefore, even though the actual plasma concentrations of the synthetic sex hormones may be similar to or even lower than the endogenous steroids, their biological action may be much stronger.

Synthetic sex hormones may be metabolized differently and have different half-lives to endogenous steroids. Although synthetic sex hormones may have certain effects in common with the endogenous hormones, their action may not be identical in all respects.

1.8-The effects of estrogen on serum biochemical parameters:

1.8.1-Glucose concentration:



Glucose

Glucose according to (Murray *et al*., 2003) is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose, and other sugars are converted into glucose in the liver. Glucose is the

major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus. It is the precursor for synthesis of all the other carbohydrates in the body, including glycogen for storage; ribose and deoxyribose in nucleic acids; and galactose in lactose of milk, in glycolipids, and in combination with protein in glycoproteins and proteoglycans.

A study done on male rabbits demonstrated that plasma glucose level was not changed due to injection of estradiol valerate (Nematbakhsh *et al.*, 2001). While there is study on overectomized rats showed that 17β- estradiol lowered glucose because of raising insulin level (Verma *et al* ., 2005), however (Nagira *et al* ., 2006) found that 17β- Estradiol inhibited the insulin and increased glucose level. A number of studies have suggested that estrogens have a profound modulating effect on systemic glucose homeostasis (Barros *et al* ., 2009, and Foryst-Ludwig and Kintscher, 2010). However a data from population studies showed that estrogen was associated with lower fasting glucose and insulin levels (Espeland *et al* .,1998). On the other hand the endocrine pancreas is not considered a classic estrogen target, but estrogen receptors are present in islets of Langerhans (Nadal *et al.*, 2000)and the effects of 17 β -estradiol in some physiological aspects of the islet of Langerhans have been known for a long time (Sutter-Dub , 2002).

1.8.2-Total protein:

Total protein is a biochemical test aiming to measure the total amount of protein in serum or plasma . plasma proteins function as; sources of amino acids, maintain the colloid osmotic pressure, influence the suspensions of RBCs, regulate acid-base balance, affect the solubility of the substances held in solution in plasma, and transport substances bounded by plasma proteins included sex steroids (Reece, 2005). Estrogen causes positive nitrogen balance due to growth promoting effect which causes slight increase in the total body proteins (Indu, 2009). 17β - estradiol

was found to be decrease total protein (Stevenson *et al* ., 2005). While a study in Red Sea bream fish *Chrysophrys major* demonstrated that total protein was elevated due to administration of Estradiol-17β (Woo *et al.,* 1993).

1.8.3-Albumin:

Albumin, is the major serum protein, binds a wide variety of lipophilic compounds including steroids, other lipophilic hormones and phytochemicals that bind to hormone receptors. However, due to albumin's high concentration in serum, albumin is the major carrier of steroids , lipophilic hormones and regulator of their access to receptors (Baker, 1998). The albumin synthesis occurs in the liver (Reece, 2005). The impact of exogenous estrogens on the liver is dependent on the route of administration and the type and dose of estrogen (Schoultz *et al.,* 1989). Oral administration of synthetic estrogens has profound effects on liver-derived plasma proteins, whereas parenteral administration of native estradiol has very little influence on these aspects of liver function (Schoultz *et al* ., 1989). In Red Sea bream fish *Chrysophrys major* 17-β estradiol elevate serum albumin (Woo *et al.,* 1993).

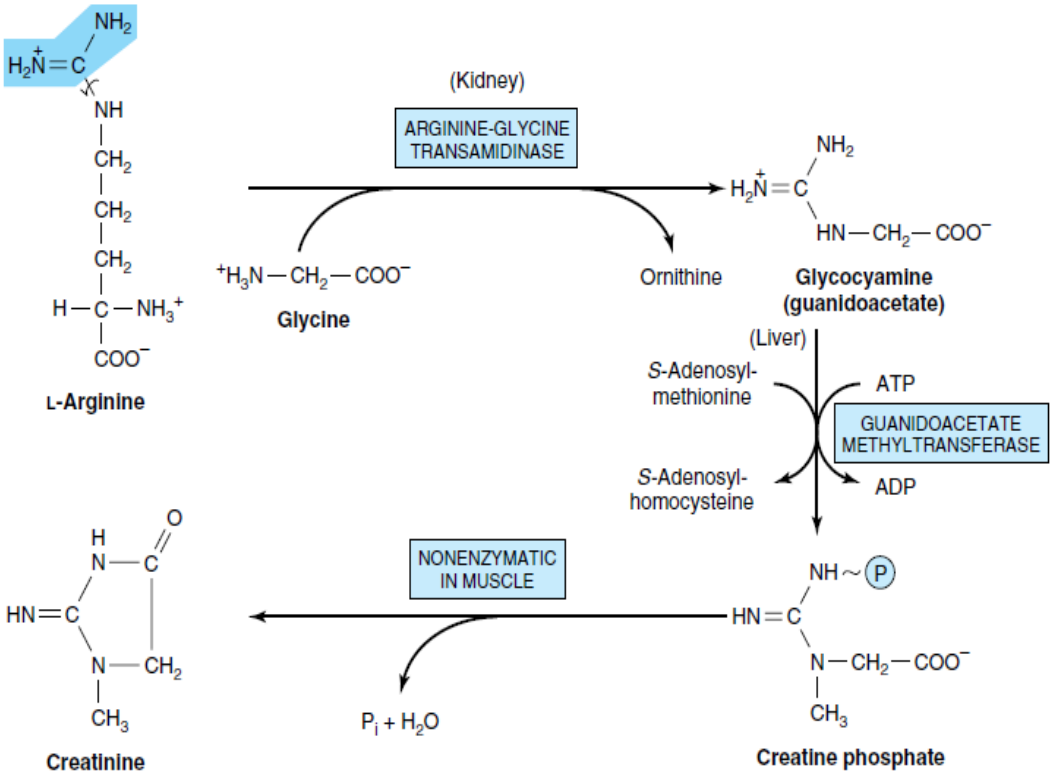
1.8.4-Calcium:

Calcium plays important roles in cellular structural and biochemical functioning through signaling within and external to the cell, as well as calcium is essential for normal neuromuscular function and for correct functioning of the coagulation factors. It is the most prevalent cation in the body and is found in the skeleton, soft tissue, and extracellular fluid (Bazydlo *et al* ., 2014). Calcium concentration in plasma is regulated by parathyroid hormone, vitamin D and calcitonin (Bazydlo *et al* ., 2014). A study on postmenopausal osteoporosis found that estrogen treatment increases calcium absorption by increasing serum 1,25-

(OH)2D, and this effect appears to be mediated indirectly through stimulation of renal alpha-hydroxylase by increased serum PTH (Gallagher *et al.*, 2013). A study done in Tilapia, *Oreochromis mossambicus* demonstrate that estrogen (E2) has a hypercalcemic effect on both males and females, with this effect being greater in males (Tsai and Wang, 2000).

1.8.5-Creatinine:

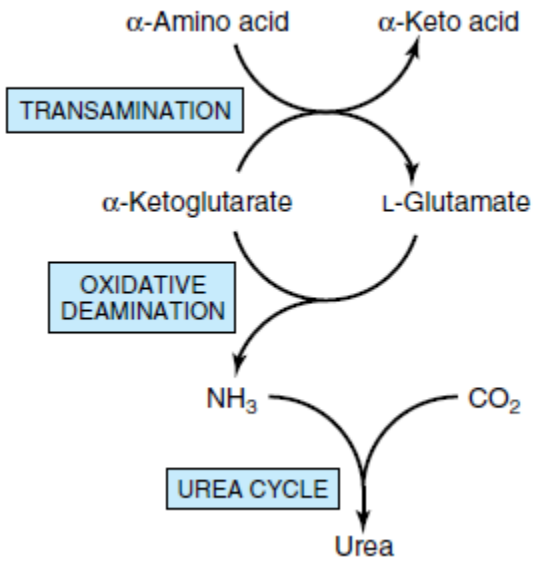
Creatinine is a waste product of protein in the diet and the muscles of the body. Creatinine is removed from the body by the kidneys. Creatinine is formed in muscle from creatinine phosphate by irreversible, non-enzymatic dehydration and loss of phosphate (Murray *et al*., 2003). A study in male mice fed with estrogen 10 μg/mice/day by (Islam, 2013), found that no significant change was observed in Creatinine.



Biosynthesis and metabolism of creatinine and Creatine phosphate. From Harper's Illustrated Biochemistry (Murray *et al*., 2003).

1.8.6-Urea:

Urea is the major end product of nitrogen metabolism. Urea biosynthesis occurs throw these steps: transamination, oxidative deamination of glutamate, ammonia transport, and reactions of the urea cycle.The effect of estrogen in urea was investigated by (Islam, 2013), and found that no significant changed in blood urea of male mice fed estrogen.



Biosynthesis of Urea. from Harper's Illustrated Biochemistry (Murray *et al.*, 2003).

1.8.7-Cholesterol, High Density Lipoprotein (HDL) Low Density Lipoprotein (LDL) , very Low Density Lipoprotein (VLDL) and Triglycerides:

Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and the outer layer of plasma lipoproteins. It is synthesized from acetyl-CoA in many tissues and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. Cholesterol is present in tissues and plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester. In plasma, both forms are transported in lipoproteins. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver,

where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as reverse cholesterol transport (Murray *et al*., 2003).

According to (Murray *et al*., 2003), there are four major groups of lipoproteins have been identified which are plays an important roles in physiology and clinical diagnosis. These are: chylomicrons, produced from intestinal absorption of triacylglycerol and other lipids; very low density lipoproteins (VLDL, or pre-β-lipoproteins), produced from the export of triacylglycerol in liver; low-density lipoproteins (LDL, or β- lipoproteins), representing a final stage in the catabolism of VLDL; and high-density lipoproteins (HDL, or α-lipoproteins), involved in VLDL and chylomicron metabolism and also in cholesterol transport.

Triacylglycerols are the major energy-storing lipids, synthesized by progressive acylation of glycerol 3-phosphate (Murray *et al*., 2003).

A considerable data are documented that in postmenopausal estrogen cause an increase in HDL and reduction of LDL (Pagnini-Hill *et al.*, 1996). Other studies have established that estrogen decrease total plasma cholesterol and increase or maintains plasma triglycerides (Miller *et al.*, 1991,Farish *et al.*, 1996, and Landenpara *et al.*, 1996).

There was a study on the effect of estrogen in mice (male and female) by (Islam, 2013); the mice were fed with estrogen 10 μg/mice/day. The study found that, HDL was increased in male but LDL, Cholesterol, and Triglycerides had not shown any changes. estrogens, particularly when unopposed by progesterone, can increase triglyceride levels through reduced triglyceride-clearing enzymes and increased endogenous triglyceride synthesis (Mantel-Teeuwisse *et al.*, 2001).

1.8.8-Aspartate Aminotransferase (AST) ,Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP):

AST and ALT are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys, it can be used in combination with other enzymes to monitor the course of various liver disorders (Xing-Jiu *et al.,* 2006). Alkaline phosphatase ALP activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in bone (osteoblasts), liver, and placenta. Elevations in serum ALP activity commonly originate from the liver and bone (Burtis *et al.,* 2008).

The effect of estrogen on serum enzymes AST and ALT, in male mice fed with estrogen 10 μg/mice/day by (Islam, 2013), found significant increase serum enzymes AST and ALT. Estrogen as is known inhibitor of the potential for thyroid hormone (PTH), particularly in its work. However, in female used oral contraceptives containing estrogen, the PTH activity is inhibited and ALP being significantly reduced (Stock *et al.,* 1985). On the other hand (Smith, and Sizto ,1983) have noted increased ALT and AST with lowered ALP activities during three months treatment of oral contraceptives.

1.8.9 - Luteinizing hormone (LH) and follicle-stimulating hormone (FSH):

Luteinizing hormone (LH), also called interstitial-cell stimulating hormone (ICSH), and follicle-stimulating hormone (FSH) are two gonadotropic hormones (*i.e.,* hormones concerned with the regulation of the gonads, or sex glands) that is produced by the pituitary gland. LH is a glycoprotein and operates in conjunction with follicle-stimulating hormone (FSH).

Estrogen play a physiological role in regulation of the reproductive functions of mammalian including the regulation of gonadotropin feedback (Carreau , 2003).

Due to it is biosynthesis occurs in the testes, and also because the absence of its receptors in most parts of male reproductive system, estrogen is said to play a regulatory role in the male reproductive tract because estrogen causes adverse effects on both spermatogenesis and steroidogenesis (O'Donnell *et al*., 2001).

Endogenous estradiol-17β exerts negative feedback effects on the secretion of gonadotropins FSH and LH, affecting the hypothalamo-pituitary system (Bellido *et al* ., 1990; Sharpe and Skakkebaek , 1993; and Sharpe *et al* ., 1998). The estradiol has a direct effect on developing spermatids, as in rats, the oral dose of exogenous estradiol sufficient to induce sterility (Biegel *et al* ., 1998). However, in the male rat, intra-peritoneal doses as low as 10 ng estradiol/rat were reported to induce morphological lesions and spermatogenic arrest without affecting gonadotropin release, after 21 dai1y exposures (Seegers *et al* ., 1991).

1.8.10-Testosterone:

Testosterone is C19 steroid, together with the more potent 5 α-reduced metabolite dihydro testosterone (DHT), it is responsible for male development in *utero,* and secondary sexual characteristic and male reproductive function and fertility at puberty and in adult life (McEwan, 2004).

A study on the direct effects of estradiol on Leydig cell testosterone biosynthesis in hypophysectomized rats by (Kalla *et al.,* 1980) found that estradiol *in vivo* can directly interfere with Leydig cell function and the principal manifestation of the inhibitory effect of estradiol on testosterone biosynthesis is an apparent reduction in 17–20 desmolase activity.

1.9-The Accessory sex glands:

Accessory sex glands in male are including the ampullar, vesicular , prostate and bulbourethral glands (Rowen *et al* .,2009).

1.9.1 -Functions of accessory sex glands:

The male accessory sex glands produce the semen, and it is important component of the male reproductive system, which functions to provide favorable condition of nutrition, buffer against the natural female genital tract acidity and medium for sperm transport (Rowen *et al* .,2009).

1.9.2-Species variation in accessory sex glands:

There is species variation in which glands are present or not as shown in the

The species variation in accessory sex glands

	Ampullary	Bulbourethral	Prostate	Vesicular
Boar	-	+	+	+
Dog	-	-	+	-
Tom Cat	-	+	+	-
Bull	+	+	+	+
Stallion	+	+	+	+
Buck	+	+	+	+
Ram	+	+	+	+
Rabbit	-	+	+	+

Ram and Buck have disseminated part of prostate gland only.

1.9.3-Prostate gland:

The prostate gland develops from the urogenital sinus (UGS) and its growth and development begin in fetal life and it complete at sexual maturity (Marker *et al*., 2003). The growth of the mammalian prostate is controlled by androgens (McPherson *et al*., 2001). The development, growth, and function of the prostate gland are regulated by the actions of multiple hormones and growth factors (Nakonechnaya *et al* ., 2013).

1.9.3.1-General function of prostate gland:

The prostate gland is an exocrine gland found in all mammals. It secretes substances essential for the normal function of the spermatozoa, include enzymes, amines, lipids and metal ions, Accumulation and secretion of extraordinarily high levels of citrate is one of the principal functions of the prostate gland of humans and other animals (Costello and Franklin, 1989).

1.9.3.2-The arrangement of the prostatic lobes:

The concepts of lobation of the prostate in animals were based solely upon gross examination. The functional lobation of the prostate is also necessary on the basis of differing responsiveness of hormones of the glandular tissue located ventral to the urethra as compared to those situated dorsally(Cole and Cuppus,1959). Presence of three paired lobes in the prostate gland of rat was dorsocranial, dorsolateral and ventral (Cole and Cuppus,1959 and Hebel and Stomberg,1976). There were two lateral lobes in the prostate gland and absence of seminal vesicles in the dog (Cole and Cuppus, 1959 and Sisson,1962). There is single lobed dorsal prostate and paired seminal vesicle in prairie dogs (Wells,1935, and Anthony,1953). Presence of four lobes in the prostate gland of rabbit was anterior, posterior and a pair of lateral (Parker and Haswell,1982).

1.9.3.3- The prostate gland of male rabbits:

The rabbit prostate gland according to (Holtz and Foote ,2005, and McCracken and Kainer , 2008) is complex, constructed in three separated parts , pro-prostate, prostate and Para-prostate. The pro-prostate part is situated caudally to the vesiculous gland and cranially to the prostate. The prostate and Para-prostate parts are situated cranially to the bulbourethral glands. These glandular parts are connected ventrally with the middle of the pelvic urethra, and laterally with the colliculus seminalis.

1.10- The effect of hormones on prostate gland:

1.10.1-Estrogen:

Administration of exogenous estrogen in adult rodents was found to cause squamous metaplasia of the anterior prostate lobe as reported by (Risbridger *et al.*., 2001 and Risbridger *et al*., 2001), a study done on alpha ERKO and beta ERKO mice by (Prins *et al* ., 2001) demonstrated that the exposure of neonatal rodents to high doses of estrogen is known to permanently imprint the growth and function of the prostate and predispose the gland to hyperplasia and severe dysplasia analogous to prostatic intraepithelial neoplasia with aging. Estrogen-induced aberrations in prostate epithelial growth have also been observed in dogs, monkeys, and humans (Coffey and Walsh 1990, and Prins and Korach 2008). Several transgenic mouse models have recently been established for use in prostate studies.

1.10.2-Testosterone:

The development and maintenance of normal prostatic structure and function is androgen dependent (Bentel and Tilley , 1996). Removal of androgen supply lead to prostate gland atrophy and involution result to epithelial cell

apoptosis (Montalvo *et al*., 2000, McConnell , 1990, 1995). Other studies showed that bio-available prostatic testosterone levels decline with age ,and DHT activity was reported higher in BPH relative to normal prostate gland tissue resulting as a permissive, rather than a transformative, mediator in the development of BPH. (Roberts *et al.*, 2004). Moreover, analysis of cadaver specimens showed an increased accumulation of DHT in BPH tissues (Siiteri and Wilson, 1970, and Geller *et al.*, 1976). Conversely, other authors reported no differences in DHT pattern when fresh specimens of prostate tissue were used (Walsh *et al.*, 1983). The transcription of a number of mitogenic growth factors in epithelial and stromal cells of prostate is an increase by androgens, this factor can act in an autocrine and / or paracrine on the epithelium to regulate cell growth, differentiation and apoptosis (Ware,1994, Farnsworth, 1999).

1.10.3-Prolactin:

Prolactin (PRL) is mainly synthesized and secreted by the lactotrop cells of the pituitary (Freeman *et al*., 2000), but also by extra-pituitary sites such as mammary gland, placenta, uterus and T lymphocytes (Bentel and Tilley , 1996).

The Prolactine hormone is synthesizing and secretes by lactotrophic cells in the anterior pituitary gland under control of multiple regulators. These can broadly be classified as endocrine, paracrine, juxtacrine or autocrine,depending on their respective origin. The secretory activity of the lactotrophs reflects a balance between local and distant inhibitory and releasing factors. In the absence of target gland hormones to provide feedback control over the lactotrophs, PRL also to some extent auto regulates its own release (Devost and Boutin, 1999). In the hypothalamus, PRL interacts with the dopaminergic systems. Dopamine has long been attributed a dominant role as an inhibitor of PRL secretion by acting itself as the main PRL inhibiting factor (PIF) (Horowski and Graf 1976).

PRL and its receptor are expressed in human and rat prostate epithelial cells, where their level is increased by androgen treatment (Nevalainen *et al*., 1997a), it has been also viewed as an autocrine/paracrine growth factor (Nevalainen *et al*., 1997b) or a survival factor (Ahonen *et al*., 1999) for the prostate epithelial cells in vitro.

1.11- Role of prolactin in mediation of estrogen effects:

Estrogens are known to stimulate growth of pituitary lactotrophs (Lloyd , 1983 and Perez *et al* ., 1986). Recent studies explained that the estradiol is a major stimulator of prolactin secretion by increasing the number of lactotrophs (Kansra *et al*., 2005, 2010, Nolan and Levy ,2009). Prostate growth, differentiation and function are primarily controlled by androgens but estrogens modulate these effects in several ways by indirect and direct mechanisms (Harkonen and Makela, 2004) . The indirect estrogen regulation are interference of androgen production by repression of the hypothalamic-pituitary-gonadal axis and direct effects on testis and the indirect estrogen regulation of prostate is via prolactin. estrogen appear to be induced hyperprolactinemia as an important mediator of estrogen action in prostate carcinogenesis (Tam *et al*., 2010).

Besides androgens, prostatic actions of estrogens are closely associated with those of prolactin (Harkonen , 2003) either pituitary-derived prolactin or prostate epithelium-produced endogenous prolactin (Nevalainen *et al*.,1997b). It is thus possible that prolactin mediates estrogen effects in prostate at the systemic and cellular level as an important mechanism in estrogen-facilitated development of dysplastic changes in prostate (Harkonen , 2003).

1.12-Orchidectomy(surgical castration):

Orchidectomy is a technical term given to the surgical removal of the testis of the male although it can also apply to spaying or removal of the ovaries of female as well (Frandson, 1981).

The effects of bilateral orchidectomy on serum protein and enzyme levels were investigated in West African Dwarf bucks . this study was found that the activity of alkaline phosphatase was increased numerically, there was a significant drop ($p<0.05$) in the activity of Aspartate amino transferase in the last 2 weeks of the study while alanine transaminase (ALT) showed variation which were in some cases significant, and a slight hypoproteinaemia, which was traceable mainly to the globulin fraction(Oyeyemi *et al.,* 2000). A preliminary study on the effect of castration on testosterone level in the New Zealand male rabbit, found that, the plasma level of testosterone of the rabbits in the bilaterally castrated group did not return to the pre-castration level, and there was a slight increase in the level of plasma testosterone observed in the second and third weeks of the experiment and this may have been due to the production of testosterone by the adrenal cortical cells of the adrenal gland (Sanni *et al* .,2012).

Chapter Two

2-Materials and Methods

2.1. Experimental animals:

Ninety adult male rabbits (54 intact and 36 castrated) weighing 2.2±0.2 kg at the beginning of the experiments, were used in this study. The rabbits were allowed 30 days for an adaptation period before each of the experiments. The animals were housed under individual cage 50 x 50 cm in groups according to the experiment plan for study objectives. They were fed on a standard diet, given water *ad libitum* and maintained daily.

2.2. Experimental design:

The animals in this study were grouped according to the objectives that were being investigated.

2.2.1. Experiment I: was aimed to study the effect of administration of different Estradiol Benzoate doses on some blood constituents and prostate gland tissue of intact rabbits. In this study thirty six intact rabbits were being used. The animals were divided into minor subg-roups according to the doses of estrogen injected as follows:

Sub groups	No of animals	Doses of estrogen in µg/ head
1	10	40
2	10	80
3	10	120
4	6	0

2.2.2. Experiment II: was aimed to study the effect of different Estradiol Benzoate doses administration on some biochemical parameters and prostate gland tissue of castrated rabbits, in this experiment thirty six castrated male rabbits were used and divided into the following sub- groups:

Sub groups	No of animals	Doses of estrogen in µg/ head
1	10	40
2	10	80
3	10	120
4	6	0

2.2.3. Experiment III: This experiment aimed to study whether the Prolactin can mediate the action of estrogen in prostate gland of intact rabbits. Prolactin secretion was inhibited by Bromocriptine Mesilate. In this study eighteen intact rabbits were being used, divided in into small groups according to treatment were being done as follows:

Sub groups	No of animals	Type of treatment
1	6	40µg estradiol + 1mg Bromocreptine /head
2	6	80µg estradiol + 1 mg Bromocreptine/ head
3	6	120µg estradiol + 1 mg Bromocreptine/ head

2.3- Estrogen administration protocols:

The estrogen which was used in this study was Estradiol Benzoate 2mg/ml (Estradol ® Animal health care Australia).

2.3.1. - Calculation of doses

Estradiol Benzoate doses were prepared according to the volume of insulin syringe; the volume of insulin syringe is 1ML divided into 100 units, into fifty lines, so each line equal 20 µgm

2.3.2. - Route of administration :

For each experiment the doses of estradiol benzoate were injected Intramuscular (IM) using insulin syringe each alternative day over a period of a month. And the injection was done after shaving the hair and application of 70% ethanol alcohol.

2.4-Inhibition of Prolactin secretion:

Prolactine secretion was inhibited by Bromocriptine Mesilate 2.5 mg tablet Brameston® , Cyprus (EU). A tablet of 2.5 mg was dissolved in 2.5 ml normal saline (NS) each ml contain 1 mg Bromocriptine. In experiment III, each rabbit was drenched 1mg of Bromocriptine in 1 ml normal saline per oral (PO) every alternative day over a period of 30 days .

2.5- The castration proses:

The testicles were being removed surgically and the aim of castration was to eliminate the testosterone secretion by Leydig cells of the testes. The general anaesthesia was induced using the protocol of (Ketamine:Xylazine) in dose of

20mg/kg:2.5mg/kg IM. After surgical operations the animals were injected antibiotic (penicillin) to prevent secondary bacterial infection.

2.6- Collection of samples:

2.6.1- Blood collection:

At the end of each experiment, a five ml of blood was being collected into plastic containers using disposable syringe 22X $1\frac{1}{4}''$ from the heart, and the serum samples were being harvested into Eppendorf tubes, deep-frozen for later analysis.

2.6.2- Prostate tissue collection:

All the animals were being slaughtered at the end of each experiment and the prostate tissue samples were collected into vial containing 10% formal saline.

2.7- Analysis of samples:

2.7.1- Serum analysis:

Serum samples were analyzed for assessing the level of blood constituents (Glucos, total-protein, albumin, calcium, urea, creatinine, lipid profiles, enzymes AST, ALT, and ALP, hormones FSH, LH, and testesterone).

2.7.1.1. Glucose:

Serum glucose concentration was measured using glucose oxidase / peroxidase methods according to (Trinder, 1969), automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made were used.

2.7.1.1.1. Principle of the method:

$$\text{Glucose} + \frac{1}{2}O_2 + H_2O \xrightarrow{glucose\ oxidase} \text{Gluconate} + H_2O_2.$$

$$2H_2O_2 + \text{4-Aminantipyrine} + \text{phenol} \xrightarrow{peroxidase} \text{Quinoneimine} + 4H_2O.$$

The intensity of the Quinoneimine color is proportional to glucose concentration in the sample.

2.7.1.1. 2. The components of reagent:

Reagent	Components
A Reagent	Phosphate 100 mmol/L, phenol 5 mmol/L, glucose oxidase > 1 U/mL, 4-aminantipyrine 0.4 mmol/L , pH 7.5
S Reagent	Glucose standard 100 mg/ dL

2.7.1.2. Total protein:

Serum total protein concentration were measured using Biuret reaction methods according to (Gornall *et al*.,1949),automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made were used.

2.7.1.2.1. Principle of the method:

Protein in the samples react with the copper (II) ion in alkaline medium forming a colored complex that can be measured by spectrophotometry (Gornall *et al*.,1949).

2.7.1.2.2. The composition of reagents:

Reagent	Components
A Reagent	Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.
S Reagent	Total protein standard 65.8 g/ L

2.7.1.3.Albumin:

Serum albumin concentration was measured using Bromocresol green methods according to (Doumas *et al*.,1971), automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made were used.

2.7.1.3.1 Principle of the method:

Albumin in the samples reacts with Bromocresol green in acid medium forming a colored complex that can be measured by spectrophotometry.

2.7.1.3. 2. The composition of reagents:

Reagent	Components
A Reagent	Acetate buffer 100 mmol/L, Bromocresol green 0.27 mmol/L, detergent, pH 4.1.
S Reagent	Albumin standard 50.9 g/ L

2.7.1.4. Calcium:

Serum calcium concentration was measured using O-Cresolphthalein complexone methods according to (Lorentz,1982),automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made were used.

2.7.1.4. 1 Principle of the method:

Calcium in the samples reacts with O-Cresolphthalein complexone (O-CPC) forming a colored complex that can be measured by spectrophotometry.

2.7.1.4. 2. The composition of reagents:

Reagent	Components
A Reagent	Ethanolamine 900 mmol/L
B Reagent	O-Cresolphthalein complexone 0.3 mmol/L, 8-hydroxyquinoline 28 mmol/L, hydrochloric acid 100 mmol/L
S Reagent	Calcium standard 10 mg/ dL

2.7.1.5. Creatinine:

Creatinine in the serum was measured by alkaline picrate method according to (Fabiny and Ertingshausen ,1971). automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made were used.

2.7.1.5.1. Principle of the method:

Creatinine reacts weith picrate in alkaline medium forming a coloured complex mesured spectrophotometry.

2.7.1.5. 2. The composition of reagents:

Reagent	Components
A Reagent	Sodium hydroxide 0.4 mol/ L, detergent
B Reagent	Picric acid 25 mmol/L
S Reagent	Creatinine 2 mg/dl

2.7.1.6. Urea:

Serum urea concentration was measured using Urease/Salicylate methods according to (Chaney and Marbach , 1962, Searcy *et al.*, 1967 , and Tabacco *et al.*, 1979). Automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made was used.

2.7.1.6.1. Principle of the method:

$$\text{urea} + 2H_2O \xrightarrow{urease} 2NH4^+ + Co2$$

$$NH4^+ + \text{Salicylate} + \text{NaCLO} \xrightarrow{nitroprusside} \text{Indophenol}$$

Indophenol is a colored complex that can be measured spectrophotometry.

2.7.1.6. 2. The composition of reagents:

Reagent	Components
A1 Reagent	Sodium salicylate 62mmol/L, sodium nitroprusside 3.4 mmolg/L, phosphate buffer 20 mmol/ l, pH 6.98
A2 Reagent	Urease > 500 U/ Ml
B Reagent	Sodium hypochlorite 7 mmol/L , Sodium hydroxide 150 mmol/L
S Reagent	Urea 50 mg/dL

2.7.1.7. Cholesterol:

Serum cholesterol concentration was measured using cholesteroloxidase/Peroxidase methods according to (Allain *et al* ., 1974, and Meiattini *et al.*, 1978). Automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made was used.

2.7.1.7. 1 Principle of the method:

Cholesterol ester + H2O $\xrightarrow{cholesterol\ esterase}$ cholesterol + fatty acid

Cholesterol + $\frac{1}{2}\ O_2$ + H2O $\xrightarrow{cholesterol\ oxidase}$ cholestenone +H2O2

2H2O2 + 4-Aminoantipyrine + phenol $\xrightarrow{peroxidase}$ Quinoneimine + 4H2O

Quinoneimine is a colored complex that can be measured spectrophotometry.

2.7.1.7. 2. The composition of reagents:

Reagent	Components
A Reagent	Pipes 35mmol/L, sodium cholate 0.5 mmolg/L, phenol 28 mmol/ l, cholesterol esterase >0.2U/mL, cholestrol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-Aminoantipyrine 0.5 mmol/L , pH 7.0
S Reagent	Cholesterol 200 mg/dL

2.7.1. 8. High Density Lipoprotein (HDL) :

High Density Lipoprotein (HDL) concentration was measured after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the sample with phosphotungstate and magnesium ions as described by (Grove, 1979, and Burstein *et al.*, 1980). Automate chemistry analyzer (MINDARY) at 500nm, and the reagent *Biosystem*® Spain made was used.

2.7.1.8.1 Principle of the method:

Cholesterol ester + H2O $\xrightarrow{cholesterol\ esterase}$ cholesterol + fatty acid

Cholesterol + $\frac{1}{2}\ O_2$ + H2O $\xrightarrow{cholesterol\ oxidase}$ cholestenone +H2O2

2H2O2 + 4-Aminoantipyrine + phenol $\xrightarrow{peroxidase}$ Quinoneimine + 4H2O

Quioneimine is a colored complex that can be measured spectrophotometry.

2.7.1.8. 2. The composition of reagents

Reagent	Components
Precipitating reagent	Phosphotungstate 0.4 mmol/L, magnesium chloride 20 mmol/L
Standard reagent	HDL cholesterol 15 mg/ dL

2.7.1.8. 3. Additional reagents:

Reagent	Components
Cholesterol Reagent	Pipes 35mmol/L, sodium cholate 0.5 mmolg/L, phenol 28 mmol/ l, cholesterol esterase >0.2U/mL, cholestrol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-Aminoantipyrine 0.5 mmol/L , pH 7.0

2.7.1.9. Precipitation of (VLDL) (LDL) in the sample:

Precipitations were done using phosphotungstate and magnesium ions (precipitating reagent), and centrifugation as described by (Grove, 1979, and Burstein *et al.,* 1980). 0.2 ml of sample and 0.5 ml of A-reagent were pipette into labelled tubes and centerifugated at 4000 r.p.m for 10 minutes , and the supernatants were carefully collected.

2.7.1.10 . Low Density Lipoprotein (LDL):

Low density lipoprotein in the serum was precipitated with polyvinyl, and the concentration was calculated from the different between the serum total cholesterol and the cholesterol on the supernatant after centrifugation as describe by (Assmann *et al* ., 1984)

2.7.1.10.1. Principle of the method:

Cholesterol ester + H2O $\xrightarrow{cholesterol\ esterase}$ cholesterol + fatty acid

Cholesterol + $\frac{1}{2}\ O_2$ + H2O $\xrightarrow{cholesterol\ oxidase}$ cholestenone +H2O2

2H2O2 + 4-Aminoantipyrine + phenol $\xrightarrow{peroxidase}$ Quinoneimine + 4H2O

Quioneimine is a colored complex that can be measured spectrophotometry.

2.7.1.10. 2. The composition of precipitating reagent:

Reagent	Components
A Reagent	Polyvinyl sulphate 3g/L, polyethyleneglycol 3 g/L

2.7.1.10. 3 Additional reagents :

Reagent	Components
Cholesterol Reagent	Pipes 35mmol/L, sodium cholate 0.5 mmolg/L, phenol 28 mmol/ l, cholesterol esterase >0.2U/mL, cholestrol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-Aminoantipyrine 0.5 mmol/L , pH 7.0
Standard	Cholesterol 200 mg/dL

2.7.1.10.4. Precipitation procedure :

0.2 ml of serum samples and 0.2 ml of precipitating reagent were pipette into labelled tubes, mixed and let stand for 15 minutes at room temperature, then were centerifugated at 4000 r.p.m for 15 minutes, and the supernatants were carefully collected.

2.7.1.11 . Triglycerides:

Triglycerides in the serum samples were measured by glycerol phosphate oxidase/ peroxidase methods according to (Bucolo and David , 1973, and Fossati and Prencipe, 1982). At 500nm using automate chemistry analyzer (MINDARY), and the reagent *Biosystem*® Spain made .

2.7.1.11.1. Principle of the method:

Triglycerides + H2O $\xrightarrow{lipase}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{glycerol\ kinase}$ Glycerol-3-P + ADP

Glycerol-3-P + O_2 $\xrightarrow{\text{Glycerol}-3-\text{P}-\text{oxidase}}$ Dihidroxyacetone + 4H2O2

2H2O2 + 4-Aminoantipyrine + 4-chlorophenol $\xrightarrow{peroxidase}$ Quioneimine +4H2O

Quioneimine is a colored complex that can be measured spectrophotometry.

2.7.1.11. 2. The composition of reagents:

Reagent	Components
A Reagent	Pipes 45 mmol/L, magnesium chloride 5 mmol/ L, 4-cholorophenol 6mmol/L, lipase > 100U/mL, glycerol kinase > 1.5 U/mL, peroxidase > 0.8 U/mL, 4-Aminoantipyrine 0.75 mmol/L , ATP 0.9 mmol/L, pH 7.0
S Reagent	Glycerol 200mg/dL

2.7.1.12. Enzymes:

2.7.12.1. Aspartate Aminotransferase (AST):

AST was measured by the method described by (Gella *et al* ., 1985 and IFCC, 1986)

2.7.1.12.1.1. Principle of the method:

Aspartate aminotransferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm by means of the malate dehydrogenase (MDH) couple reaction (Gella *et al* ., 1985 and IFCC, 1986).

$$\text{Aspartate + 2-Oxoglutarate} \xrightarrow{AST} \text{Oxalacetate + Glutamate}$$

$$\text{Oxalacetate + NADH + } H^{+} \xrightarrow{MDH} \text{Malate + } NAD^{+}$$

2.7.1.12.1.2. The composition of reagents:

Reagent	Components
A Reagent	Tris 121mmol/L, L-aspartate 362 mmol/ L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660U/L, sodiumhydroxide 255 mmol/L pH 7.8
B Reagent	NADH 1.9 mmol/L, 2-Oxoglutrate 75 mmol/L, sodiumhydroxide 148 mmol/L, sodium azide 9.5 g/L
Auxiliary reagents	Pyridoxal phosphate 10 mmol/L

2.7.1.12.2 . Alanine Aminotransferase (ALT):

ALT was measured by the method described by (Gella *et al* ., 1985 and IFCC, 1986).

2.7.12.2 .1. Principle of the method:

Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate forming pyruvate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm by means of

the lacate dehydrogenase (LDH) couple reaction (Gellaq *et al* ., 1985 and IFCC, 1986).

$$\text{Alanine + 2-Oxoglutarate} \xrightarrow{ALT} \text{Pyruvate + Glutamate}$$

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{LDH} \text{Malate + NAD}^{+}$$

2.7.12. 2. 2. The composition of reagents

Reagent	Components
A Reagent	Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3
B Reagent	NADH 1.9 mmol/L, 2-Oxoglutrate 75 mmol/L, sodiumhydroxide 148 mmol/L, sodium azide 9.5 g/L

2.7.1.12.3 . Alkaline phosphatase (ALP):

Alkaline phosphatase was measured using 2-Amino-2-Methyl-1-Propanol buffer method according to (IFCC , 2011)

2.7.1.12.3.1. Principle of the method:

Alkaline phosphatase (ALP) catalyzes in alkaline medium, the transfer of phosphate group from 4-nitrophenylphosphatase to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation (IFCC , 2011).

2.7.1.12.3.2. The composition of reagents:

Reagent	Components
A Reagent	2-amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylenediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L pH 10.4
B Reagent	4-Nitrophenylphosphate 60 mmol/L.

2.7.1.13. hormones FSH, LH, and Testosterone:

Serum FSH, LH, and testosterone levels were performed by immunoenzymometric assay (mouse anti-LH monoclonal antibodies, mouse anti-FSH monoclonal antibodies and mouse anti-Testosterone monoclonal antibodies; TOSOH Corporation Japan) in an automatic analyzer TOSOH AIA TOSOH Corporation Japan).

2.7.2- Tissues analysis:

2.7.2.1. Prostate histological change study:

Preparation of tissues for histological examination was carried out according to (Bancroft *et al.*,1996),as follow:

2.7.2.1.1.Fixation:

About one cm^3 of prostate tissue specimens were collected from control and estrogen treated groups immediately by using a sharp knife or a razor blade, and were fixed immediately in 10% formal saline, the volume ratio of tissue to fixative was 1:10. The tissue was left at room temperature for fixation before they were processed.

2.7.2.1.2.Tissue processing:

Tissue processing method used was made by automatic tissue processor (**LEICATP1020)**.The scheduled program followed was: 65% alcohol, 70% alcohol, 80% alcohol, 95% alcohol and two changes of absolute alcohol respectively. Cleared by chloroform two changes followed by xylene one change, and impregnated with two changes of paraffin wax at 60^oC. The specimens were

then embedded in fresh wax, left to solidity at room temperature followed by refrigeration to solidify the blocks enough for microtomy.

2.7.2.1.3. Microtomy:

The paraffin-embedded tissue blocks were cut in 5µ thick sections by a rotary microtome, the sections were mounted on labelled slides and dried at room temperature for 24 hours.

2.7.2.1.4. Staining with H&E:

Before the staining process all tissue sections were treated as follow:

- Dewaxed with xylene for 5 min (two changes).
- hydrated with descending alcohol concentrations; for two min in each of 3 change of absolute alcohol followed by two changes in 90% and 70% alcohol for three min in each concentration .
- The sections were then transferred to distilled water for two minutes and then stained by Mayer's haematoxylin as follow:

Staining in Mayer's haematoxylin for 7-10 min, differentiation in 1% acid alcohol washed in running tap water for 10 min and stained by 1% Eosin for 3-5 min. the sections were then dehydrated cleared and mounted in DPX.

2.7.2.1.5. Microscopic examination:

All sections were examined under the light microscope (Olympus) to describe the microscopic changes and imaged by using digital camera (Dewinter- DigiEye).

2.8. Statistical analysis:

All data obtained from level of blood constituents were statistically analyzed using SPSS statistical program, version 20 for Windows (IBM SPSS Statistics 20 IL, USA). The results were expressed in the form of mean ± standard deviation. The difference between the mean in level of serum biochemical parameters in this study were considered statistically significant when the *P* value was less than 0.05.

CHAPTER THREE

3- Results

3.1. The effects of Estradiol Benzoate on blood constituents of intact male rabbits:

3.1.1. Concentration of Glucose, Total-protein, Albumin, Calcium, Urea, and Creatinine:

The effects of Estradiol benzoate on concentration of glucose, T-protein, albumin, calcium, urea, and creatinine were presented in Table (2). There was a significant ($p<0.05$) increase in total protein in the 40 μg Estradiol treated sub-group and significant ($p<0.05$) increase in glucose concentration in the 80 μg Estradiol treated sub-group. On the other hand there was significant ($p<0.05$) decrease in glucose concentration in the 40 μg Estradiol treated sub-group, glucose and Creatinine in the 120 μg Estradiol treated sub-groups, compared to 0μg Estradiol treated sub-group. However there was no significant difference ($p>0.05$) between 40 μg, 80 μg , 120μg and 0μg Estradiol treated sub- groups in albumin, calcium urea, total protein between 80 μg, 120 μg and 0μg Estradiol treated sub-groups, Creatinine between 40 μg, 80 μg and 0μg Estradiol treated sub-groups.

Table (1). The effect of estradiol benzoate in concentration of Glucose, T-protein, Albumin, Globulin, Calcium, Urea, and Creatinine of intact males rabbits:

Parameters	0 µg Estradiol	40 µg Estradiol	80µg Estradiol	120 µg Estradiol
Glucose mg/dL	88.50± 3.11	75.50± 9.11*	96.75± 1.59*	37.25±1.03*
T- protein g/L	06.30± 1.05	06.53± 0.15*	05.76± 0.56	06.13±0.34
Albumin g/L	04.53± 0.29	4.40± 0.08	04.28±0.36	04.40± 0.04
Calcium mg/dL	14.33± 0.68	16.48± 0.96	14.40± 1.07	14.38± 0.62
Urea mg/dL	60.00± 1.17	70.75± 1.58	56.75± 7.93	79.50± 1.24
Creatinine mg/dL	01.43± 0.51	01.13± 0.43	01.05± 0.30	01.18± 0.15*

- Means with superscript stars within the row were significantly different ($P<0.05$), compared to 0 µg Estradiol treated sub-group when t-test was used.
- Data were presented in form of mean ± SD

  ❖ SD ≡ standard deviation.

3.1.2. Lipids profile:

The effects of Estradiol benzoate on lipids profiles were presented in Table (3). There was significant ($p<0.05$) increase in HDL in the 40 µg Estradiol treated sub-group. On the other hand there was insignificant ($p>0.05$) decrease in triglycerides and LDL in 40 µg, 80 µg and 120 µg Estradiol treated sub-groups compared to 0 µg Estradiol treated sub-group. However there was no significant difference ($p>0.05$) between 40 µg, 80 µg , 120 µg and 0 µg Estradiol treated sub-groups in cholesterol, and HDL between 80 µg , 120 µg and 0 µg Estradiol treated sub-groups.

Table (2). The effects of estradiol benzoate on Lipids profile of intact males rabbits:

Parameters	0 µg Estradiol	40µgEstradiol	80µg Estradiol	120 µg Estradiol
Triglycerides mg/dL	58.25± 2.58	23.25± 1.71	10.75± 1.71	13.00±1.41
Cholesterol mg/dL	28.25± 6.08	29.25± 7.23	19.25± 4.79	29.00± 1.81
HDL mg/dL	09.89± 2.79	16.73± 5.69*	12.08±3.05	12.78± 5.39
LDL mg/dL	13.25± 3.30	07.50± 1.73	03.25± 1.50	03.45± 0.64

- Means with superscript stars within the row were significantly different ($P<0.05$), compared to 0 µg Estradiol treated sub-group when t-test was used.
- Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.1.3. AST, ALT and ALP Enzymes of intact male rabbits:

The response of AST, ALT and ALP enzymes to different estradiol benzoate doses were presented in Table (4). There was significant ($p<0.05$) increase in ALT in the 120 µg Estradiol treated sub-group, in significant ($p>0.05$) increase in ALT in the 80 µg Estradiol treated sub-group , in significant ($p>0.05$) decrease in ALP between 40 µg, 80 µg , 120 µg and 0 µg Estradiol treated sub-groups. However there were no significant ($p>0.05$) changes in AST between 40 µg, 80 µg , 120 µg and 0 µg Estradiol treated sub-groups.

Table (3). The effects of estradiol benzoate on AST, ALT and ALP Enzymes of intact male rabbits:

	0µg Estradiol	**40 µ Estradiol**	**80µg Estradiol**	**120 µg Estradiol**
AST	72.75± 2.31	51.75± 4.43	55.25± 1.25	82.25±4.80
ALT	65.75± 1.10	56.50± 3.51	120.00± 3.16	118.00± 6.77 *
ALP	77.00± 5.88	27.75± 7.23	36.25±6.65	41.50± 1.88

- Means with superscript stars within the row were significantly different ($P<0.05$), compared to 0 µg Estradiol treated sub-group when t-test was used.
- Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.1.4. FSH, LH and Testosterone hormones of intact male rabbits:

The data were present on table (5). There was significant (P< 0.05) increase in testosterone in 120 µg Estradiol treated sub-group, insignificant (P> 0.05) increase of testosterone in 40 µg and 80 µg Estradiol treated groups. However there were no significant (p>0.05) changes in FSH and LH between 0 µg and 40 µg, 80 µg and 120 µg Estradiol treated sub-groups.

Table (4). The effects of Estradiol benzoate on FSH, LH and testosterone hormones of intact males rabbits:

Parameters	0µg Estradiol	40 µg Estradiol	80µg Estradiol	120 µg Estradiol
FSH mIU/mL	00. 55± 0.31	00.68± 0.23	00.55± 0.16	00.86± 0.12
LH mIU/mL	00.12± 0.06	00.09± 0.04	00.04± 0.02	00.06± 0.03
Testosterone ng/ml	17.00± 8.32	60.14± 1.22	42.75± 2.81	63.94± 2.41*

• Means with superscript stars within the row were significantly different (P<0.05), compared to 0 µg Estradiol treated sub-group when t-test was used.

• Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.2. The effects of Estradiol Benzoate on biochemical parameters of castrated rabbits:

3.2.1. Concentration of Glucose, Total-protein, Albumin, Calcium, Urea, and Creatinine:

The effects of different estradiol benzoate doses on serum Concentration of Glucose, T-protein, Albumin, Calcium, Urea, and Creatinine biochemical parameters were presented in Table (6). There was significant ($p<0.05$) increase of glucose in the 40 μg, 80μg and 120 μg Estradiol treated sub-groups and significant ($p<0.05$) increase of urea concentration in the 80 μg and 120 μg Estradiol treated sub-groups. On the other hand there was significant ($p<0.05$) decrease of total-protein concentration in the 40 μg, 80 μg, and 120 μg Estradiol treated sub-groups, and Creatinine in the 40 μg and 120 μg Estradiol treated sub-groups. However there was no significant difference ($p>0.05$) between 40 μg, 80 μg and 120 μg and 0 μg Estradiol treated sub-groups in albumin and calcium, urea between 40 μg and 0 μg Estradiol treated sub-groups and Creatinine between 80 μg and 0 μg Estradiol treated sub- groups.

Table (5). Concentration of Glucose, Total-protein, Albumin, Calcium, Urea, and Creatinine of castrated males rabbits:

Parameters	0µg Estradiol	40 µ Estradiol	80µg Estradiol	120 µg Estradiol
Glucose mg/dL	88.50±03.11	143.50± 21.83*	155.50± 03.87*	143.50± 25.32*
T- protein g/L	06.30± 1.05	05.89± 0.13*	05.58± 0.17*	05.73±0.34 *
Albumin g/L	04.53± 0.29	04.28± 0.13	04.08±0.17	04.35± 0.21
Calcium mg/dL	14.33± 0.68	14.40± 0.24	13.85± 0.19	13.90± 0.64
Urea mg/dL	60.00± 1.17	60.00± 5.29	75.75± 4.35*	72.00± 5.24*
Creatinine mg/dL	01.43± 0.51	01.23± 0.10*	00.90± 0.37	00.98± 0.17*

• Means with superscript stars within the row were significantly different ($P<0.05$), compared to 0 µg Estradiol treated sub-group when t-test was used.

• Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.2.2. Lipids profile :

The data were shown on table (7). There was no significant (P>0.05) differences in means of lipids profiles between 40 µg, 80 µg ,120 µg and 0µg Estradiol treated sub-groups, despite the presence of insignificant increase on HDL and decrease on triglycerides of 40 µg, 80 µg and 120 µg Estradiol treated su-groups, and LDL in 80 µg Estradiol treated sub-group.

Table (6). The effects of Estradiol benzoate on Lipids profile of castrated male rabbits:

	0 µg Estradiol	**40 µg Estradiol**	**80µg Estradiol**	**120 µg Estradiol**
Triglycerides mg/dL	58.25± 2.58	14.25± 1.26	13.50± 1.73	15.00± 8.68
Cholesterol mg/dL	28.25± 6.08	34.25± 4.79	25.00± 4.08	24.25± 3.30
HDL mg/dL	09.89± 2.79	17.35± 4.12	11.83±4.60	14.75± 2.47
LDL mg/dL	13.25± 3.30	12.00± 1.41	04.50± 1.29	09.50± 1.00

No significant differences in means (P>0.05) when t-test was used.

• Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.2.3. AST, ALT, and ALP Enzymes :

The data were present on table (8). There was significant (P>0.05) decrease in AST of 40 µg and 80 µg Estradiol treated sub-groups and ALP on 40 µg, 80 µg and 120 µg Estradiol treated sub-groups. On the other hand there was no significant changes between 40 µg, 80 µg and 120 µg and 0 µg Estradiol treated sub-groups in ALT, and AST in 120 µg Estradiol treated sub-groups.

Table (7). The effects of Estradiol benzoate on AST, ALT and ALP Enzymes of castrated males rabbits:

	0 µg Estradiol	40 µg Estradiol	80µg Estradiol	120 µg Estradiol
AST IU/mL	72.75± 2.31	28.00± 2.94*	24.75± 3.50*	40.25±7.04
ALT IU/mL	65.75± 1.10	77.50± 2.04	47.00± 1.87	80.25± 1.06
ALP IU/mL	77.00± 5.88	17.50± 3.11*	14.50± 1.29*	13.00± 0.82*

- Means with superscript stars within the row were significantly different (P<0.05) when t-test was used.

• Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

3.2.4. FSH, LH, and Testosterone Hormones :

The data were shown on table (9). There was no significant (P>0.05) differences in means of gonadotropins (LH, FSH) and testosterone hormones between 40 µg, 80 µg , 120 µg and 0 µg Estradiol treated sub-groups, despite the presence of increase on testosterone hormone of 40 µg, 80 µg and 120 µg Estradiol treated sub-groups.

Table (8). The effects of Estradiol benzoate on FSH, LH and testosterone hormones of castrated rabbits:

	0 µg Estradiol	**40 µg Estradiol**	**80µg Estradiol**	**120 µg Estradiol**
FSH mIU/mL	00. 55± 0.31	00.85± 0.08	00.79± 0.14	00.75± 0.13
LH mIU/mL	00.12± 0.06	00.11± 0.04	00.09± 0.02	00.57± 0.31
Testosterone ng/mL	17.00± 8.32	59.79± 1.08	58.50± 1.31	58.58± 5.89

• No significant differences in means (P>0.05), when t-test was used.

• Data were presented in form of mean ± SD.

∴ SD ≡ standard deviation.

4 - The correlation between estradiol benzoate doses and blood biochemical parameters:

4. 1- Intact male rabbits:

The data of correlation were shown in table (10), there was significant positive correlation in ALT ($r = 0.56$) ($P<0.02$), testosterone ($r = 0.54$) ($P<0.03$), and significant negative correlation in glucose ($r = -0.59$) ($P<0.02$), triglyceride ($r = 0.75$) ($P<0.00$), LDL ($r = -0.64$) ($P<0.00$), and LH ($r = -0.56$) ($P <0.03$). insignificant ($P>0.05$) positive correlation in urea ($r = 0.36$), HDL ($r = 0.10$), AST ($r = 0.13$), FSH ($r = 0.39$). insignificant ($P>0.05$) negative correlation in total protein ($r = -0.24$), albumin ($r = -0.25$), creatinine ($r = -0.26$), calcium ($r = -0.18$), cholesterol ($r = -0.09$), and ALP ($r = -0.33$).

Table (9). Correlation between estradiol benzoate doses and biochemical parameters of intact male rabbits:

	Parameters	R	*P* value
	Glucose	-0.59	0.02
	Total-protein	-0.24	0.38
	Albumin	-0.25	0.35
	Urea	0.36	0.17
	Creatinine	-0.26	0.33
	Calcium	-0.18	0.50
	Cholesterol	-0.09	0.75
Dose	Triglyceride	-0.75	0.00
	HDL	0.10	0.71
	LDL	-0.64	0.00
	AST	0.13	0.62
	ALT	0.56	0.02
	ALP	-0.33	0.21
	FSH	0.39	0.13
	LH	-0.56	0.03
	Testosterone	0.54	0.03

4.2- Castrated male rabbits:

The data of correlation were shown in table (11), there was significant positive correlation in glucose ($r = 0.82$) ($P<0.00$) , urea ($r = 0.54$) ($P<0.03$) and testosterone ($r = 0.85$) ($P<0.00$). And significant negative correlation in creatinine ($r = -0.53$) ($P <0.03$), triglyceride ($r = -0.80$) ($P <0.00$), AST ($r = -0.73$) ($P <0.00$), and ALP ($r = -0.70$) ($P <0.00$). Insignificant ($P>0.05$) positive correlation in HDL ($r = 0.40$), ALT ($r = 0. 07$), FSH ($r = 0.47$) and ($r = 0.09$). Insignificant ($P>0.05$) negative correlation in total protein ($r = -0.47$), albumin ($r = -0.46$), calcium ($r = -0.34$), cholesterol ($r = -0.23$), and LDL ($r = -0.28$).

Table (10). Correlation between estradiol benzoate doses and biochemical parameters of castrated male rabbits:

	Parameters	*R*	*P* value
	Glucose	0.82	0.00
	Total-protein	-0.47	0.07
	Albumin	-0.46	0.07
	Urea	0.54	0.03
	Creatinine	-0.53	0.03
	Calcium	-0.34	0.20
	Cholesterol	-0.23	0.40
Dose	Triglyceride	-0.80	0.00
	HDL	0.40	0.13
	LDL	-0.28	0.30
	AST	-0.73	0.00
	ALT	0.07	0.80
	ALP	-0.70	0.00
	FSH	0.47	0.07
	LH	0.44	0.09
	Testosterone	0.85	0.00

5- The effect of Estradiol on prostate glands tissue:

5.1 - Intact males rabbits:

The effect of Estradiol on prostate glands tissue of intact male rabbits was presented in the figure.1, the microphotograph A demonstrated the normal histology of the prostate tissues of 0µg Estradiol group. While the microphotograph (B,C and D) 40, 80 and 120µg Estradiol treatment groups demonstrated the presence of hyperplastic (*short arrows*) and dysplastic (*long arrows*) lesions.

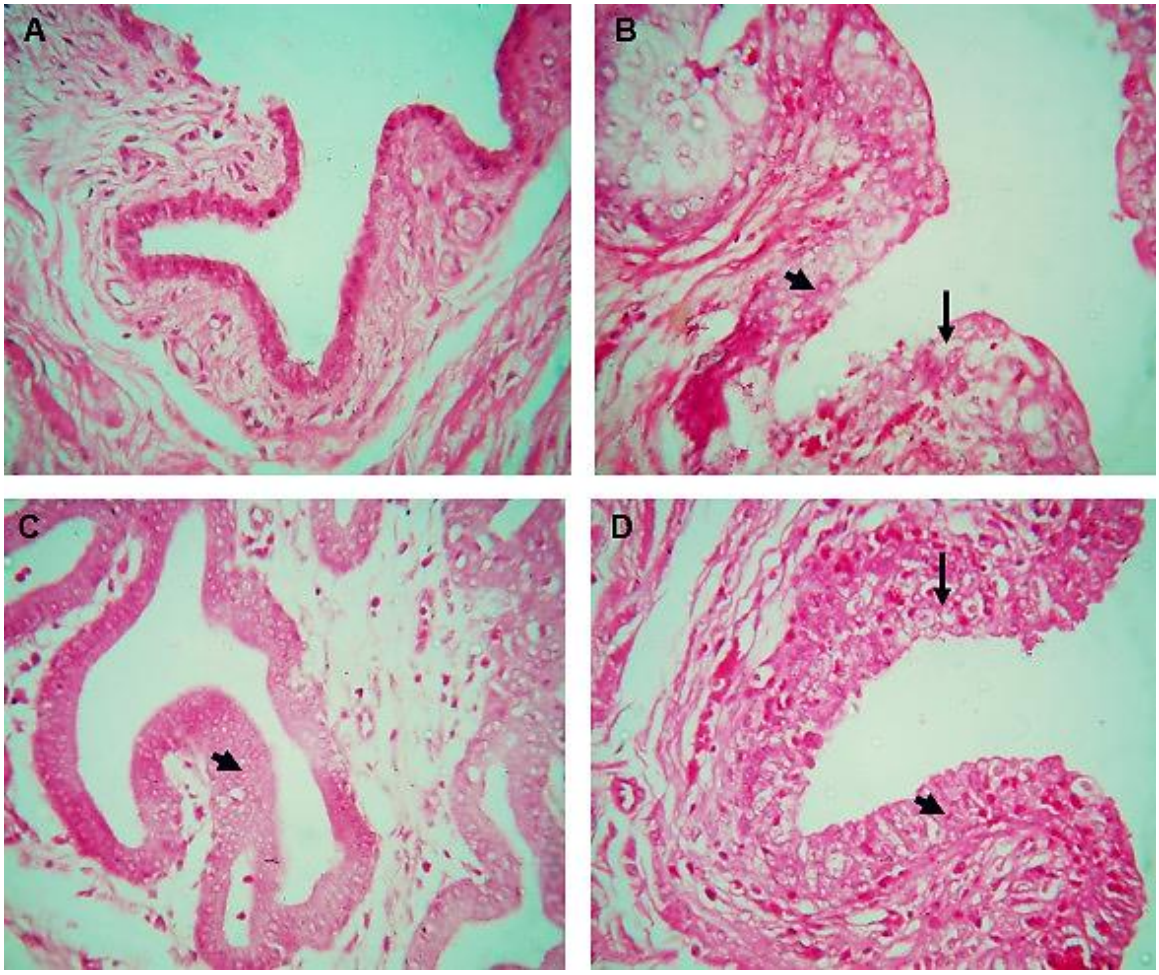


Figure 1. A microphotograph of Estradiol-treated rabbit prostate tissues, magnification ×400. (A) No obvious histological change was observed in the prostate tissues of the 0µg Estradiol treatment group. (B-D) 40, 80 and 120µg estradiol treatment sub-groups demonstrating the presence of hyperplastic (*short arrows*) and dysplastic (*long arrows*) lesions.

5.2- Castrated males rabbits:

The effect of Estradiol on prostate glands tissue of castrate male rabbits was presented in the figure.2, the microphotograph A demonstrated the normal histology of the prostate tissues of 0µg Estradiol treatment sub-group. While the microphotograph (B-D) 40, 80 and 120µg Estradiol treatment sub-groups were demonstrated the presence hyperplasia representing by papillary projection.

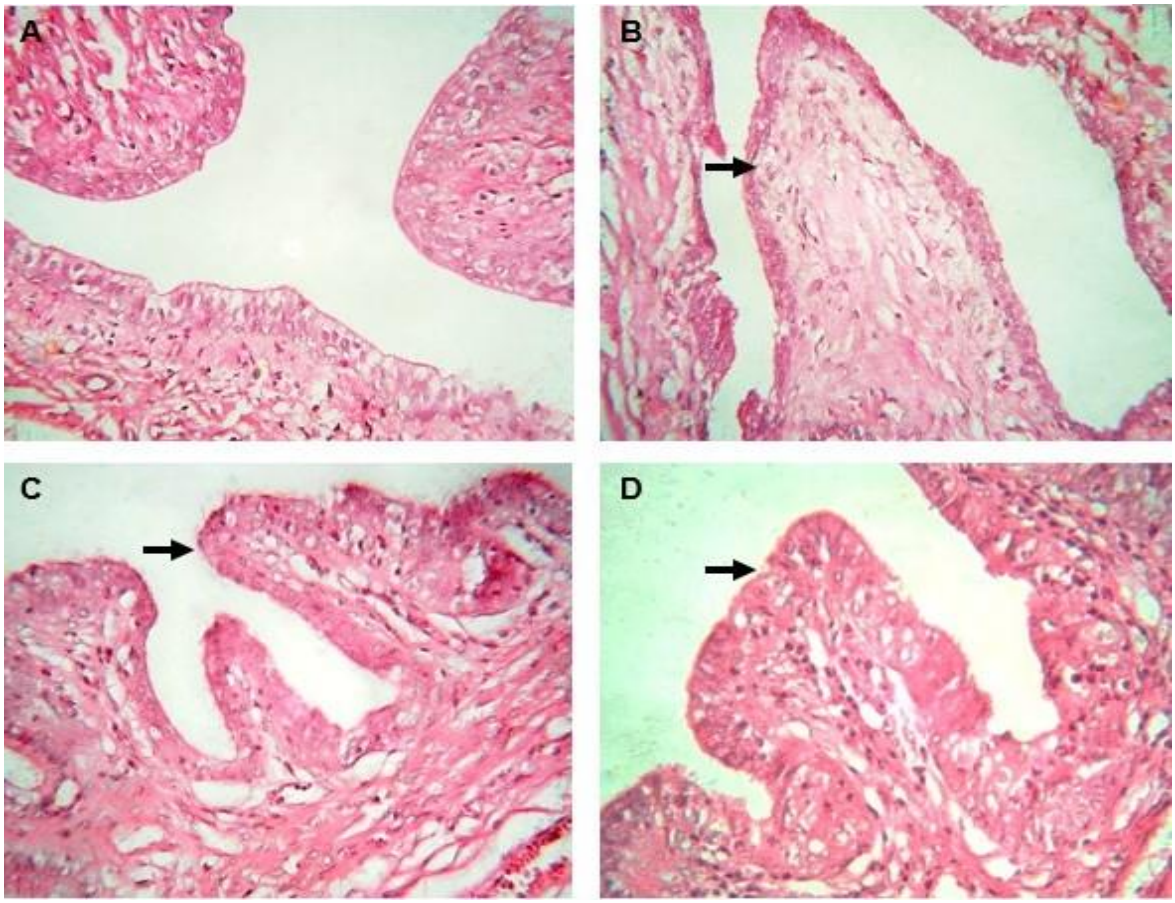


Figure 2. A microphotograph showed the effects of Estradiol on castrated-rabbit's prostate gland, magnification ×400. (A) Representative H&E of prostate section of 0µg Estradiol treatment group showed no histological changes. (B-D) 40, 80 and 120µg Estradiol treatment groups showing the presence of hyperplasia representing by papillary projection (*arrow*) in epithelia.

5.3- The effect of Estradiol benzoate on prostate tissue of intact males rabbits in presence of Prolactin inhibitor:

In the figure .3 the microphotograph A demonstrated the normal histology of the prostate tissues of 0µg Estradiol treatment group. While the microphotograph (B,C and D) 40, 80 and 120µg Estradiol treatment groups demonstrated the presence of mild hyperplasia (*arrow heads*) in epithelia.

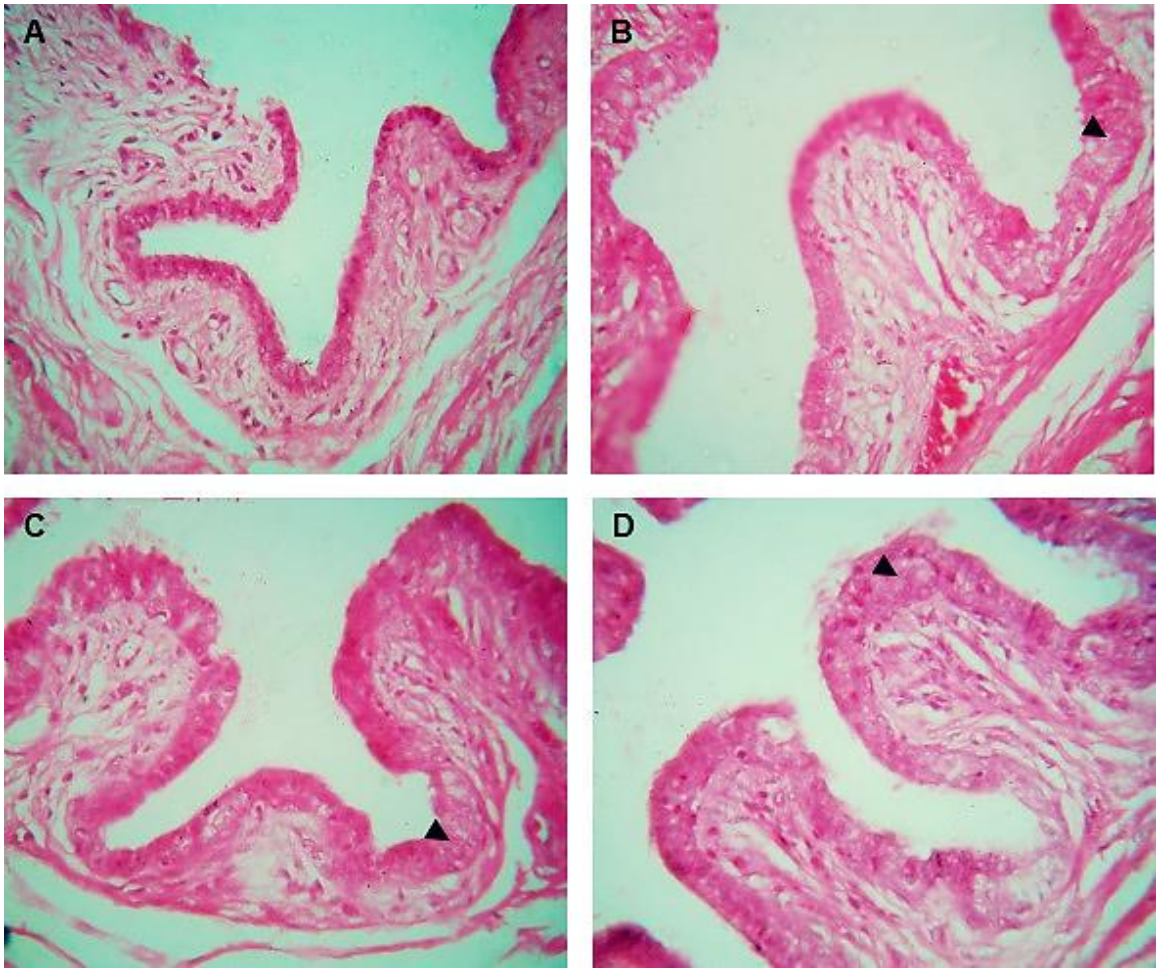


Figure 3. A microphotograph showed the effects of Estradiol in present of prolactin inhibitor (Bromocreptein mysaylate) on the histological changes in rabbit's prostate gland, magnification ×400. (A) Representative H&E of prostate section of 0µg Estradiol treatment group showed no histological changes. (B-D) 40, 80 and 120µg Estradiol treatment sub-groups showing the presence of mild hyperplasia (*arrow heads*) in epithelia.

CHAPTER FOUR

Chapter Four

4- Discussion

The differences between the action of natural and synthetic steroid hormones must be but in considers as (Anna-Maria and Niels ,1999) mentioned that many synthetic sex steroids have a low affinity for sex hormone binding globulin (SHBG) and are present in an unbound form and biologically active in plasma. In contrast, approximately 98–99% of the endogenous sex steroids are bound to SHBG in plasma. Therefore, even though the actual plasma concentrations of the synthetic sex hormones may be similar or even lower than the endogenous steroids, their biological action may be much stronger.

The effect of Estradiol on glucose concentration in previous studies were heterogeneous. A study done on male rabbits demonstrate that plasma glucose level was not changed due to injection of estradiol valerate (Nematbakhsh *et al.*, 2001). While another study on overectomized rats showed that 17β- estradiol lowered glucose because of raising insulin level (Verma *et al* ., 2005), also a data from population studies showed that estrogen was associated with lower fasting glucose and insulin levels (Espeland *et al* .,1998). however (Nagira *et al* ., 2006) found that 17β- Estradiol inhibited the insulin and increased glucose level. On the other hand a number of studies have suggested that estrogens have a profound modulating effect on systemic glucose homeostasis (Barros *et al* ., 2009, and Foryst-Ludwig and Kintscher, 2010).

In the current study the result of glucose response to injection of Estradiol benzoate in intact rabbits were ranged from significant ($p<0.05$) increase in glucose concentration in the 80 μg Estradiol treated sub- group to significant ($p<0.05$) decrease in the 40 and 120 μg Estradiol treated group, and significant

($p<0.05$) increase of glucose in the 40 µg, 80µg and 120 µg Estradiol treated sub group of castrated rabbits. This finding was supported by several researchers; (Nagira *et al* ., 2006) found that in adipocytes increase in glucose level due to inhibition of insulin level by the 17β- estradiol, while in rat (Verma *et al* ., 2005) showed that 17β- estradiol lowered glucose because of raising insulin level.

Estrogen were found to cause a positive nitrogen balance due to growth promoting effect which causes slight increase in the total body proteins (Indu, 2009), while (Stevenson *et al* ., 2005 and Elnagar and Abd-Elhady ,2009) found that 17β - estradiol decrease total protein. A study in red sea bream *Chrysophrys major* found that total proteins were elevated due to administration of Estradiol-17β (Woo *et al.*, 1993). The total-protein levels in this study were found to be significantly ($P<0.05$) increased in 40 µg estradiol treated sub-group of intact male rabbits, but slightly as similar result was reported by (Indu, 2009). And, the increasing of total-protein was disagreed with the findings of (Stevenson *et al*., 2005and Elnagar and Abd-Elhady,2009) who found decreased level of total-protein. On the other hand, insignificantly ($P>0.05$) change in total-protein was shown in 80 µg, 120 µg estradiol treated sub-groups of intact male rabbits; these results were disagreed with the studies of (Stevenson *et al.*, 2005; Elnagar and Abd-Elhady, 2009 and Indu, 2009). While the significant ($p<0.05$) decrease in the 40 µg, 80 µg, and 120 µg Estradiol treated sub-groups of castrated rabbits were agreeing with the similar of (Woo *et al.*, 1993).

Due to its high concentration in serum, albumin is the major carrier of steroids and lipophilic hormones and regulator of their access to their receptors (Baker, 1998). The albumin synthesis occurs in the liver (Reece, 2005). And the impact of exogenous estrogens on the liver is dependent on the route of

administration and the type and dose of estrogen (Schoultz *et al.*, 1989). The albumin concentration in this study was not significantly changed (P>0.05) in 40 μg, 80 μg and 120 μg Estradiol treated sub-groups of intact and castrated male rabbits; this result differed from the report of (Elnagar and Abd-Elhady ,2009). This variation could be attributed to the doses of Estradiol benzoate which were used in this study might had not any effect on albumin because the impact of exogenous estrogen on the liver (which synthesis the albumin) is dependent on the route of administration and the type and dose of estrogen (Schoultz *et al* ., 1989).

The influence of estradiol on calcium in the present study were not significantly (p>0.05) changed in 40 μg, 80 μg and 120 μg Estradiol treated sub-groups of intact and castrated male rabbits. A similar study done in fish by (Tsai and Wang ,2000) showed that the increase in serum calcium levels were dose-dependent in both gonadectomized male and female fishes. Based on the above studies, the effect of Estradiol benzoate on serum calcium might be dependent on dose of Estradiol benzoate, and these doses used in this study might not be quite enough to induce change in calcium concentration.

The effect of Estradiol on urea concentration were found to be significantly (p<0.05) increase in the 80 μg and 120 μg Estradiol treated sub-groups of castrated rabbits, and not changed (p>0.05) in 40 μg, 80 μg and 120 μg Estradiol treated sub-groups of intact and in 40 μg sub-group of castrated male rabbits . There was study on male mice fed with estrogen 10 μg/mice/day found that no significant change in blood urea (Islam, 2013). So the result of intact sub-groups were agreeing with that of similar of (Islam, 2013), but the result of 80 μg and 120 μg Estradiol treated sub-groups of castrated rabbits were disagreeing.

In this study there were significant ($p<0.05$) decrease in Creatinine of the 120 μg Estradiol treated sub-group of intact rabbits, and 40 μg and 120 μg Estradiol treated sub-groups of castrated rabbits. no significant difference ($p>0.05$) in Creatinine of 40 μg, 80 μg Estradiol treated sub-group of intact rabbits, and 80 μg Estradiol treated sub-group of castrated rabbits. The results of decreased Creatinine were disagreeing with the study in male mice by (Islam, 2013), which found no significant change observed in Creatinine. While the results of no significant difference ($p>0.05$) in Creatinine of 40 μg, 80 μg Estradiol treated sub-group of intact rabbits, and 80 μg Estradiol treated sub-group of castrated rabbits were agreeing with that of (Islam, 2013).

The effect of estrogen on lipids profiles were investigated on mice (male and female) by (Islam, 2013); the study found that, HDL was increase in male but LDL, Cholesterol, and Triglycerides have not shown any changes. Estrogens, particularly when unopposed by progesterone, can increase triglyceride levels through reduced triglyceride-clearing enzymes and increased endogenous triglyceride synthesis (Mantel-Teeuwisse *et al.*, 2001).

In this study there were no significant ($P>0.05$) differences in means of lipids profiles (Cholesterol , HDL , LDL, and triglycerides) between 40 μg, 80 μg and 120 μg and 0 μg Estradiol treated sub-groups of intact and castrated rabbits except HDL in the 40 μg Estradiol treated sub-group of intact rabbits shown significant ($p<0.05$) increase , this result was agreeing with (Islam, 2013), but HDL in the 40 μg Estradiol treated sub-group of intact rabbits has shown a disagreeing result with that obtained by (Islam, 2013).

There was no significant (P>0.05) changes in AST, ALT and ALP of Estradiol treated sub-groups of intact rabbits except ALT in the 120 µg Estradiol treated sub-group was significantly (p<0.05) increased . And there were significant (P<0.05) decrease in AST of 40 µg and 80 µg Estradiol treated sub-groups and ALP on 40 µg, 80 µg and 120 µg Estradiol treated sub-groups of castrated rabbits. On the other hand there were no significant(p<0.05) changes in ALT between 0 µg, 40 µg, 80 µg and 120 µg Estradiol treated sub- groups, and AST in 120 µg Estradiol treated sub- groups of castrated rabbits. increase serum enzymes ALT in the 120 µg Estradiol treated sub-group of intact rabbits was in agreement with study of (Islam, 2013), and decrease in ALP of 40 µg, 80 µg and 120 µg Estradiol treated sub-groups of castrated rabbits were agreeing with the similar studies in the effect of estrogen in oral contraceptives (Smith, and Sizto ,1983and Stock *et al.,* 1985). While the result of decreased AST and no change of ALT were in disagreement with that found by (Islam, 2013).

Estrogen play a physiological role in regulation the reproductive functions of mammalian species including the regulation of gonadotropin feedback (Carreau , 2003). estrogen also play a regulatory role in the male reproductive tract because it causes adverse effects on steroidogenesis (O'Donnell *et al*., 2001). Study in adult male rat shown that 17 beta estradiol have a dose-related effects on LH, FSH, and testosterone (Gill-Sharma *et al.,* 2001), at low dose of 0.1 µg/kg/day Estradiol have not any effects in serum level of LH, FSH and testosterone, but at high doses of 10 and 100-1000 µg/kg/day, significantly reduced serum level of LH, FSH and testosterone. However in this study there were no significant (P>0.05) changes found in FSH and LH of groups under study. While testosterone show different responses; ranged from significant (P<0.05) increase in high dose 120 µg, to insignificant (P>0.05) increase in a doses 40 µg and 80 µg of estradiol benzoate in

intact male rabbits, while in castrated male rabbits show insignificant (P>0.05) increase. The result of FSH and LH in intact and castrated groups and testosterone in intact group were agreeing with that obtained by (Gill-Sharma et al., 2001), but the result of testosterone in castrated group was disagreeing.

The effects of Estradiol in prostate of intact rabbits ranged from hyperplasia with dysplasia or dysplasia only in Estradiol treated sub-groups (Figure 1). A similar prostatic dysplasia result to Estrogen treatment was found in mice (Pylkkanen *et al.,* 1993), in Noble rat (Leav *et al.,* 1989; Lou *et al.,* 1998), and in the rodent Mongolian gerbil prostate (Wellerson *et al.,* 2008). In the other hand the effects of Estradiol in prostate of castrated rabbits showing the presence of hyperplasia represented by papillary projection (Figure.2).The hyperplasia of epithelial cells in castrated animals due to administration of Estrogen suggests that Estrogen can directly stimulate the activity of epithelial cells. These results are in agreement with several studies indicating that administration of Estrogen to castrated or hypophysectomized animals could exert a stimulatory action on prostate epithelial cells as in castrated rat (Pelletier, 2002) and castrated dogs (Rhodes *et al*., 2000).

In the present of Prolactin inhibitor Bromocriptine Mesilate, the injection of Estradiol benzoate induces mild hyperplasia (Figure.3). This result may indicate the direct effect of Estrogen in prostate due to elicited external hormone (Harkonen and Makela, 2004), and this elicited hormone could be Prolactin as a result to the Estrogen effects on pituitary lactotrophs as several studies suggest that (Kalland *et al,*.1980, Lopez *et al.,* 1984, and Khurana *et al.,* 2000;Van Coppenolle *et al.,* 2000, 2001, 2004;). Prolactin has been shown to be involved in the differentiation and proliferation of numerous tissues, including the prostate gland (Crepin *et al.,*

2007). And estrogen appears to be induced hyperprolactinemia as an important mediator of estrogen action in prostate carcinogenesis (Tam *et al.*, 2010).

5-Conclusion:

This work was concluded in the flowing points:

- Injection of Estradiol benzoate at dose of 40 μg/IM to intact rabbits induce:
  - Significant increase in total-protein, globulin and HDL, while induce significant decrease in glucose
- Injection of Estradiol benzoate at dose of 40 μg/IM to castrated rabbits induce:
  - Significant increase in glucose, significant decrease in total-protein, globulin, creatinine, AST, and ALP.
- Injection of Estradiol benzoate at dose of 80 μg/IM to intact rabbits induce:
  - Significant increase in glucose and significant decrease in globulin.
- Injection of Estradiol benzoate at dose of 80 μg/IM to castrated rabbits induce:
  - Significant increase in glucose and urea, while induce significant decrease in total-protein, globulin, AST, and ALP.
- Injection of Estradiol benzoate at dose of 120 μg/IM to intact rabbits induce:
  - Significant increase in ALT and Testosterone, significant decrease in glucose, globulin and creatinine.
- Injection of Estradiol benzoate at dose of 120 μg/IM to castrated rabbits induce
  - Significant increase in glucose and urea, significant decrease in total-protein, globulin, creatinine and ALP.
- Injection of Estradiol benzoate at dose of 40 μg/IM increase glucose concentration in castrated rabbits and decreased it in to intact rabbits, but it has increased total-protein and globulin in intact rabbits and decrease them into castrated rabbits.

- Injection of Estradiol benzoate at dose of 80 μg/IM was increase glucose and decrease globulin in both intact and castrated rabbits.
- Injection of Estradiol benzoate at dose of 120 μg/IM was increase glucose in castrated rabbits and decrease it into intact rabbits, while decrease globulin and creatinine in both intact and castrated rabbits.
- In intact male rabbits there was significant positive correlation between dose of estradiol benzoate and ALT and testosterone, significant negative correlation between dose and glucose triglyceride , LDL and LH.
- In castrated male rabbits there was significant positive correlation between doses of estradiol benzoate and glucose , urea and testosterone. And significant negative correlation in creatinine , triglyceride ,AST and ALP.
- Injection of Estradiol benzoate was induced hyperplastic and dysplastic lesion in to prostate tissue, while in castrated rabbits prostate was induced hyperplasia representing by papillary projection.
- Administration of Estradiol benzoate to male rabbits in concomitant with Prolactin inhibitor (Bromocreptein mysaylate) induced mild hyperplasia.

6- Recommendations:

This study is recommending by stablishing of more studies to tested the effect of high doses of estradiol benzoate more than those which used in this study.

7- References:

Adaikan, P.G and Srilatha, B.(2003). Oestrogen-mediated hormonal imbalance precipitates erectile dysfunction. **Int. J. Impot. Res. 15**:38-43.

Adamopoulos, D. Lawrence, D.M. Vassilopoulos, P. Kapolla, N. Kontogeorgos, L. McGarrigle, H.H. (1984). Hormone levels in the reproductive system of normospermic men and patients with oligospermia and varicocele. **J .Clin. Endocrinol. Metab**. **59**:447-452.

Ahonen, T.J., Harkonen, P.L. Laine, J. Rui, H. Martikainen, P.M. and Nevalainen, M.T. (1999) Prolactin is a survival factor for androgen-deprived rat dorsal and lateral prostate epithelium in organ culture. **Endocrinol, 140**: 5412–5421.

Akingbemi, B.T. Sottas, C.M. Koulova, A.I. Klinefelter, G.R. and Hardy, M.P.(2004). Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. **Endocrinolo. 145**:592–603.

Allain, C.C. Poon, L.S. Chan, C.S.G. Richmond, W. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol**. Clin. Chem 20**:470-475.

Anna-Maria, A. and Niels, E.S. (1999). Exposure to exogenous estrogens in food: possible impact on human development and health. **Eur. J. Endocrinol, 140**: 477-485.

Anthony, A. (1953) Seasonal reproductive cycle in the normal and experimentally treated male prairie dog. **J.Morph**, **93:**331-370.

Assmann, G. Jabs, Hu. Kohnert, U. Nolte, W. and Schriewer, H. (1984). LDL-cholesterol in blood serum following precipitation of LDL with polyvinylsulate. **Clin. Chem. Acta, 140**:77-83.

Atanassova, N. McKinnell, C. Turner, K. J. Walker, M. Fisher, J. S. Morley, M. Millar, M.R. Groome, N. P. and Sharpe, R. M. (2000) Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. **Endocrinolo**, **141**: 3898-3907.

Atanassova, N. McKinnell, C. Walker, M. Turner, K. J. Fisher, J. S. Morley, M. Millar, M.R. Groome, N. P. and Sharpe, R. M. (1999). Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood. **Endocrinolo, 140**:5364-5373.

Baker, M.E .(1998). Albumin's role in steroid hormone action and the origins of vertebrates: is albumin an essential protein?. **FEBS Letters, 439**: 9-12.

Balthazart, J. Cornil, C.A. Charlier, T.D. Taziaux, M. Ball, G.F. (2009). Estradiol, a key endocrine signal in the sexual differentiation and activation of reproductive behavior in quail. **J. Exp. Zool. A. Ecol .Genet. Physiol, 311**: 323-345.

Bancroft, J. D., Stevens, A., Turner, D.R.(1996) Theory and Practice of Histological Technique. 4th ed., Churchill Livingston, N.Y., London, San Francisco, Tokyo. Pp 99-112.

Barros, R.P. Gabbi, C. Morani, A. Warner, M. and Gustafsson, J.A. (2009). Participation of ER alpha and ER beta in glucose homeostasis in skeletal muscle and white adipose tissue. **Am. J. Physiol. Endocrinol Metab**. **297**: 124-133.

Bazydlo, L. A.L. Needham. M, and Harris,N.S. (2014). Calcium, Magnesium, and Phosphate. **Lab Medicine 45**: 44-50. Review.

Beekman, J.M. Allan, G.F., Tsai, S.Y. Tsai, M.J. and O'Malley, B.W.(1993). Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. **Mol. Endocrinol. 7**:1266-1274.

Bellido, C. Pinilla, L. Aguilar, R. Gaytan, F. and Aguilar, E.(1990). Possible role of changes in post-natal gonadotrophin concentrations on permanent impairment of the reproductive system in neonatally oestrogenized male rats. **J. Reprod. Fertil, 90**: 369-374.

Bentel, J.M. and Tilley, W.D. (1996). Androgen receptor in prostate cancer. **J. Endocrinol, 151**:1-11.

Biegel, L.B. Flaws, J.A. Hirshfield, A.N. O'Connor, J.C. Elliott, G.S. Ladics, G.S. Silbergeld, E.K. Van Pelt, C.S. Hurtt, M.E. Cook, J.C. and Frame, S.R. (1998). 90-Day feeding and one-generation reproduction study in Crl:CD BR rats with 17-estradiol. **Toxicol. Sci, 44**: 116-142.

Blake, C. A. Boockfor, F.R. Nair-Menon, J. U. Millette, C. F. Raychoudhury, S. S. and McCoy, G. L. (2004) Effects of 4-tert-octylphenol given in

drinking water for 4months on the male reproductive system of Fischer 344 rats. **Reprod.Toxicol**, **18**: 43-51.

Bucolo, G. and David, H. (1973). Quantitative determination of serum triglycerides by use of enzymes. **Clin. Chem 19**:476-482.

Bujan, L. Mieusset, R. Audran, F. Lumbroso, S. Sultan. C, (1993) Increased oestradiol level in seminal plasma in infertile men. **Hum Reprod**, **8**:74-77.

Burstein, M. Scholnick, H.R. and Morfin, R. (1980). Rapid method for the isolation of lipoprotein from human serum by precipitation with polynions. **Scand. J. Clin. Lab. Invest, 40**:583-595.

Burtis, C.A. Ashwood, E.R. and Bruns, E.R. (2008). Tietz Textbook of Fundamental of Clinical Chemistry,6th Ed, Saunders, an imprint of Elsevier, Inc P325.

Carreau, S. de Vienne, C. Galeraud-Denis, I.(2008). Aromatase and estrogen in man reproduction: a review and latest advances. **Adv. Med. Sci, 53**:139-144.

Carreau, S. Genissel, C. Bilinska, B. and Levallet, J. (1999). Sources of oestrogen in the testis and reproductive tract of the male**. Int. J. Androl**, **22**: 211-23.

Carreau, S. Lambard, S. Delalande, C. Denis-Galeraud, I. Bilinska, B. and Bourguiba, S. (2003). Aromatase expression and role of estrogens in male gonad . **Reprod .Biol .Endocrinol .11**:1-35.

Chaney, A.L. and Marbach, E.P. (1962). Modified reagents for determination of urea and ammonia . **Clin .Chem** , **8**:130-132.

Claus R., Schopper D., Hoang-Vu C.(1985) Contribution of individual compartments of the genital tract to oestrogen and testosterone

concentrations in ejaculates of the boar **Acta Endocrinol**, **109**:281-288.

Claus, R. Dimmick ,M.A. Gimenez, T. and Hudson, L.W. (1992). Estrogens and prostaglandin F2a in the semen and blood plasma of stallions. **Theriogenolo**, **38:**687-693.

Coffey, D.S. and Walsh, P.C. (1990). Clinical and experimental studies of benign prostatic hyperplasia. **Urol. Clin. North. Am. 17**:461–75.

Cole, H.H. and Cuppus P.T. (1959). Reproduction in domestic animals. **Academic Press**, **1**: 42-47. New York, London.

Costello, L.C. and Franklin, R.B. (1989). Prostate epithelial cells utilize glucose and aspartate as the carbon sources for net citrate production. **Prostate**. **15**:335-342.

Devost, D. and Boutin, J.M.(1999) Autoregulation of the rat prolactin gene in lactotrophs. **Mol. Cell. Endocrinol, 158**: 99–109.

Dorrington, J.H. and Khan, S.A. (1993). Steroid production, metabolism, and release by Sertoli cells. In The Sertoli Cell, pp 538–549. Eds L Russell and M Griswold. Clearwater: Cache River Press.

Doumas, B.T. Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. **Clin. Chim. Acta, 31**:87-96.

Driscoll, S. G. and Taylor, S. H. (1980) Effects of prenatal maternal estrogen on the male urogenital system. **Obstet Gynecol**, **56:** 537-542.

Eiler, H. and Graves, C. (1977). Oestrogen content of semen and the effect of exogenous oestradiol-17a on the oestrogen and androgen

concentration in semen and blood plasma of bulls. **J Reprod .Fertil**. **50**:17-21.

Ellem, S.J. and Risbridger, G.P. (2009). The dual, opposing roles of estrogen in the prostate. **Ann .N Y. Acad .Sci.. 1155**:174-186.

Elnagar, S.A. Abd-Elhady, A.M. (2009). Exogenous estradiol: productive and reproductive performance and physiological profile of Japanese Quail hens. **Int. J. Poult. Sci**, **8** : 634-641.

Espeland, M.A. Hogan, P.E. Fineberg, S.E. Howard, G. Schrott, H. Waclawiw, M.A. and Bush, T.L.(1998): Effect of postmenopausal hormone therapy on glucose and insulin concentrations: PEPI Investigators: Postmenopausal Estrogen/Progestin Interventions. **Diabetes Care 21**:1589–1595.

Evans, M.R. (1988). The steroid and thyroid hormone receptor superfamily, **Science 240**: 889-895.

Evanthia, D.K. Jean-Pierre, B. Linda, C.G. Russ, H. Gail, S. Prins, A.M. Soto, R. Thomas, Z. and Andrea, C.G. (2009). Endocrine disrupting chemicals: An endocrine society scientific statement. **Endocr. Rev**. **30**:293-342.

Fabiny, D.L. and Ertingshausen, G. (1971). Automated reaction-rate method for determination of serum creatinine with CentrifiChem. **Clin. Chem. 17**:696-700.

Farish, E. Spowart, K. Barnes, J.F. Fletcher, C.D. Hart, P.M. and Degen, M.M. (1996). Effect of postmenopausal hormone replacement therapy on lipoproteins including Lp(a) an LDL sub-fraction. **Atherosclerosis**, **126**:77-84.

Farnsworth, W.E. Slaunwhite, W.R. Jr. Sharma, M. Oseko, F. Brown, J.R. Gonder, M.J. Cartagena, R. (1981). Interaction of prolactin and testosterone in the human prostate. **Urol. Res. 9** :79-88.

Fisher, J. S. Turner, K. J. Brown, D. and Sharpe, R. M. (1999) Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. **Environ Health. Perspect**, **107**: 397-405.

Foryst-Ludwig, A. and Kintscher, U. (2010). Metabolic impact of estrogen signalling through ER alpha and beta. **J. Steroid. Biochem .Mol. Biol 122**: 74-81.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produce hydrogen peroxide. **Clin. Chem, 28**:2077-2080.

Frandson, R.D. (1981). Anatomy of the male reproductive system in: **Anatomy and Physiology of farm animals.** second Ed, Lea and Febiger. Great Britain, P430-444.

Franswoth, W.E. (1999). Prostate stroma: physiology. **Prostate**, **38**: 60-72.

Free, M.J. and Jaffe, R.A. (1979). Collection of rete testis fluid from rats without previous efferent duct ligation. **Biol. Reprod, 20**:269-278.

Freeman, M.E. Kanyicska, B. Lerant, A. and Nagy, G. (2000). Prolactin: structure, function, and regulation of secretion. **Physiol. Rev**. **80**:1523–1631.

Gallagher, J.C. Riggs, B.L. and Deluca, H. F. (2013). Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. **J. clinic. Endocrinolo, 51**(6): 1359-1364.

Ganjam, V.K. and Amann, R.P.(1976). Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. **Endocrinolo**, **99**:1618-1630.

Gellaq, F.J. Olivella, T. Cruz, P.M. Arenas, J. Moreno, R. Durban, R. and Gomez, J.A. (1985). A simple procedure for routine determination of Aspartate Aminotransferase and alanine aminotransferase with pyridoxal phosphate. **Clin. Chem. Acta, 153**:241-247.

Geller, J. Albert, J. Lopez, D. Geller, S. and Niwayama, G. (1976). Comparison of androgen metabolites in benign prostatic hypertrophy (BPH) and normal prostate. **J. Clin. Endocrinol. Metab**. **43**:686–688.

Gill-Sharma, M.K. Dosouza, S. Padwal, V. Balasinor, N. Aleem, M. Parte, P. Juneja, H.S. (2001). Antifertility effects of estradiol in adult male rats. **J . Endocrinol. Invest**, **24**: 598-607.

Gornall, A.G. Bardawill, C.S. and David, M.M.(1949) Determination of serum proteins by means of the Biuret reaction . **J. Biol. Chem, 177**: 751-766.

Grove, T.H. (1979). Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungastate-magnesium. **Clin. Chem, 25**:560-564.

Harkonen ,P. Makela, S.I. (2004). Role of estrogens in development of prostate cancer. **J. Steroid Biochem. Mol. Biol, 92**: 297-305.

Harkonen, P. (2003) Paracrine prolactin may cause prostatic problems, **Endocrinol, 144** :2266-2268.

Hebel, R. and Stromberg, M.W. (1976). Anatomy of the Laboratory Rat, The Williams and Wilkins Co. Baltimore U.S.A , p66-73.

Hess, R.A. and Carnes, K. (2004). The role of estrogen in testis and the male reproductive tract: a review and species comparison. **Anim. Reprod, 1**:5-30.

Holtz, W. and Foote, R. (2005): The anatomy of the reproductive system in male Dutch rabbits (Oryctolagus cuniculus) with special emphasis on the accessory sex glands. **J. Morphol. 158**: 1-20.

Horowski, R. and Graf, H.J. (1976). Influence of dopaminergic agonists and antagonists on serum prolactin concentrations in the rat. **Neuroendocrinolo**. **22**(3):273-86.

Horwitz, K.B. Jackson, T.A. Bain, D.L. Richer, J.K. Takimoto, G.S. and Tung, L. (1996). Nuclear receptor co-activators and co-repressors. **Mol Endocrinol**. **10**:1167–1177.

IFCC, (1986). Approved recommendation 1985 on IFCC method for the measurement of Catalytic Concentration of Enzymes. Part 2: IFCC method for Aspartate Aminotransferase (E.C 2.6.1.1). **J.Clin Chem Clin. Biochem, 24**:241-247.

IFCC, (2011).IFCC primary reference procedure for the measurement of catalytic activity concentration of enzymes at 37C°. part 9. Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. **Clin. Chem. Lab. Med, 49**:1439-1446.

Indu, K. (2009). Text book of medical physiology., Reprint 1st Ed, Elsevier publications , p859-860.

Islam, MDS, (2013). Effects of butter and estrogen on blood biochemistry and some selected organs in in reference to the development of obesity in mice. Bangladesh Agricultural University, The Digital Repository of

the Library materials (Thesis). http://dspace.bau.edu.bd/bitstream/123456789/1607/1/Roll%20No.%2012VPHYJJ-03M.pdf

Kalla, N.R. Nisula,B.C. Menard, R. and Loriaux, D.L. (1980). The effect of estradiol on testicular testosterone biosynthesis. **Endocrinol, 106**: 35-39.

Kalland, T. Forsberg, J.G. Sinha, Y.N. (1980). Long-term effects of neonatal DES treatment on plasma prolactin in female mice. **Endocr. Res .Commun**, **7**:157-66.

Kansra, S. Chen, S. Bangaru, M.L. Sneade, L. Dunckley, J.A. Ben-Jonathan, N. (2010). Selective estrogen receptor down-regulator and selective estrogen receptor modulators differentially regulate lactotroph proliferation. **PLoS ONE.** 5: e10060. doi: 10.1371/journal.pone.0010060.

Kansra, S. Yamagata, S. Sneade, L. Foster, L. Ben-Jonathan, N. (2005) Differential effects of estrogen receptor antagonists on pituitary lactotroph proliferation and prolactin release. **Mol.Cell. Endocrinol**, **239**:27–36

Khurana ,S, Ranmal, S, Ben-Jonathan, N. (2000). Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression. **Endocrinol, 141**:4512-4517.

Landenpara, S. Puolakka, J. Pyorola, T. Luotola, H. and Taskinen, M.R.(1996). Effect of postmenopausal estrogen/progestin

replacement therapy on LDL particles: comparison of transdermal and oral treatment regimens, **Atherosclerosis, 122**:153-162.

Leav, I. Merk, F.B. Kwan, P.W.L. Ho, S.M. (1989). Androgen-supported estrogen-enhanced epithelial proliferation in the prostate of intact Noble rats. **Prostate ,15**:23–40.

Lemazurier, E. and Seralini, G.E. (2002). Evidence for sulfates and 17beta-hydroxysteroid dehydrogenase type 1 activities in equine epididymis and uterus, **Theriogenolo**. **58**:113-121.

Levallet, J. Bilinska, B. Mittre, H. Genissel, C. Fresnel, J. and Carreau, S. (1998). Expression and immune localization of functional cytochrome P450 aromatase in mature rat testicular cells. **Biol. Reprod. 58**:919-926.

Lien, R. Cain, J. and Forrest, D.(1985). The influence of exogenous estradiol on bobwhite quail (Colinus virginianus) reproductive systems. **Comp Biochem Physiol A Com Physiol. 80**:433–436.

Lloyd, R.V.(1983). Estrogen-induced hyperplasia and neoplasia in the rat anterior pituitary gland. An immunohistochemical study. **Am. J Pathol, 113**:198–206.

Lopez, J. Ogren, L. Talamantes, F. (1984). Effects of neonatal treatment with diethylstilbestrol and 17 alpha- hydroxyprogesterone caproate on in vitro pituitary prolactin secretion. **Life Sci. 34** :2303-2311.

Lorentz, K. (1982). Improvement determination of serum calcium with 2-cresophthalein complexone. **Clin. Chem. Acta, 126**:327-334.

Lou, K.M. Leav, I. Ho, S.M. (1998). Rat estrogen receptor-apha and beta and progesterone receptor mRNA expression in various prostatic lobes

and microdissected normal and dysplastic epithelial tissues of the Noble rats. **Endocrinol, 139**:404-427.

Luboshitzky, R. Kaplan-Zverling, M. Shen-Orr, Z. Nave, R. and Herer, P (2002b) Seminal plasma androgen/ oestrogen balance in infertile men. **Int .J. Androl.** 25:345-351.

Luboshitzky, R. Shen-Orr, Z. and Herer, P. (2002a). Seminal plasma melatonin and gonadal steroids concentration in normal men. Arch. Androl. **48**:225-232.

Mantel-Teeuwisse, A.K. Kloosterman, J.M. Maitland-van der Zee, A.H, Klungel, O.H, Porsius, A.J. and de Boer A (2001). Drug-induced lipid changes: a review of unintended effects of some commonly used drugs on serum lipid levels. **Drug Saf, 24**:443-456.

Marker, P.C. Donjacour, A.A. Dahiya, R. and Cunha, G.R. (2003). Hormonal, cellular, and molecular control of prostatic development. **Dev .Biol, 253**: 165–174.

Marselos, M. and Tomatis, L. (1992) Diethylstilboestrol: I, Pharmacology, Toxicology and carcinogenicity in humans. **Eur J Cancer**, **28** :1182-1189.

McConnell, J.D (1990). Androgen ablation and blockade in the treatment of benign prostatic hyperplasia. **Urol. Clin. North. Am**, **17**: 661-670.

McConnell, J.D (1995). Benign prostatic hyperplasia: hormonal treatment. **Urol. Clin. North. Am**, **22**: 387-400.

McCracken, T.O. Kainer, R . (2008): Color Atlas of Small Animal Anatomy: The Essentials. Blackwell Publishing, USA, p 72.

McKinnell, C. Atanassova, N. Williams, K. Fisher, J. S. Walker, M. Turner, K. J. Saunders,P. T. K. and Sharpe, R. M. (2001) Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. **J. Androlo**, **22**(2) :323-338.

McLachlan, J. A. Newbold, R. R. Burow, M. E. and Li, S. F. (2001) From malformations to molecular mechanisms in the male: three decades of research on endocrine disrupters. **Acta Pathol Microbiol Immunol Scand A, 109**: 263-272.

McPherson, S.J. Wang. H, Jones, M.E. Pedersen, J. Iismaa, T.P. Wreford, N. Simpson, E.R. Risbridger, G.P. (2001). Elevated Androgens and Prolactin in Aromatase-Deficient Mice Cause Enlargement, But Not Malignancy, of the Prostate Gland . **Endocrinol** ,**142**: 2458-2467.

McWan, I.J. (2004). Molecular mechanism of androgen receptor mediated regulation: structural – functional analysis of the AF-1 domune. **Endocrine. Related Cncer, 11**:281-293.

Meiattini, F. Prencipe, L. Bardelli, F. Giannini, G. and Trali, P. (1978). The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. **Clin. Chem**, **24**:2161-2165.

Miller, V.T. Muessins, R.A. Larosa, J.C. Philips, E.A. and Stilman, R.J. (1991). Effect of conjugated estrogen with and without three different progestins on lipoprotein sub-fraction and apolipoprotein A1. **Obestric and Gynecol, 77**:235-240.

Mittendorf, R. (1995) Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. **Teratology**, **51**: 435-445.

Montalvo L, Carmena MJ, Solano RM, Clemente C, Román ID, Sánchez-Chapado M, Prieto JC. (2000). Effect of flutamide-induced androgen-receptor blockade on adenylate cyclase activation through G-protein coupled receptors in rat prostate. **Cell Signal. 12:**311-316**.**

Moskowitz, D. (2006). A Comprehensive Review of the Safety and Efficacy of Bioidentical Hormones for the Management of Menopause and Related Health Risks. **Altern. Med. Rev**. **11**: 208-223.

Murray, R.K. Granner, D.K. Mayes, P.A. and Rodwell, V.W. (2003). Harper's Illustrated Biochemistry 26th ed McGraw- Hill Companies, Inc.pp

Nadal, A. Ropero, A.B. Laribi, O. Maillet, M. Fuentes, E. and Soria, B. (2000). Non genomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor alpha and estrogen receptor beta. **Proc. Natl. Acad. Sci .USA** .**97**:11603–11608.

Naderi, A.R. and Safarinejad, M.R.(2003) Endocrine profiles and semen quality in spinal cord injured men. Clin .Endocrinol. (Oxf), **58**:177-184.

Nagira, k. Sasoaka, T. wada, T. Fukui, K. Ikubo, M. Hori, S. Tsuneki, H. Satio, S. and kobavashi, M.(2006) . Altered sub cellular distribution of estrogen receptor alpha is amplicated in estradiol –induced regulation of insulin signiling in 3T3- l1 adipocytes. **Endocrinol, 147**:1020-1028.

Nakonechnaya, A.O. Jefferson, H.S. Chen, X. Shewchuk, B.M. (2013). Differential effects of exogenous and autocrine growth hormone on

LNCaP Prostate Cancer cell proliferation and survival , **J. Cell. Biochem**. **114**:1322–1335. .

Nematbakhsh, M. Samarian, H. Sarafzadegan, N. Sharifi, M.R. Ghorvi, M. Amini, M. Mohammadifard, N. Alikhalesi, H. and Soltani, N. (2001). The effect of estrogen on plasma insulin and glucose level, and cholesterol- HDL and LDL- HDL ratio in normal and cholesterol-red male rabbits. **Pajouhesh Dar Pezeshki 25** :17-24.

Nevalainen, M.T. Valve, E.M. Ahonen, T. Yagi, A. Paranko, J. and Harkonen, P.L. (1997a). Androgen dependent expression of prolactin in rat prostate epithelium in vivo and in organ culture. **Faseb. J**, **11**:1297-1307.

Nevalainen, M.T. Valve, E.M. Ingleton, P.M. Nurmi, M. Martikainen, P.M. and Harkonen, P.L. (1997b). Prolactin and prolactin receptors are expressed and functioning in human prostate. **J. Clin. Invest, 99**:618-627.

Nilsson, S. Mäkelä, S Treuter, E. Tujague, M. Thomsen, J. Andersson, G. Enmark, E. Pettersson, K. Warner, M. Gustafsson, J.A.(2001). Mechanisms of estrogen action. P**hysiol. Rev, 81**:1535-1565.

Nitta, H. Bunick ,D. Hess, R.A. and Janulis, L. (1993) Germ cells of the mouse testis express P450 aromatase. **Endocrinol**, **132**:1396-1401.

Nolan, L.A. Levy, A. (2009). The trophic effects of oestrogen on male rat anterior pituitary lactotrophs. **J. Neuroendocrinol**, **21**:457–464

O'Donnell, L. Robertson, K.M. Jones, M.E. and Simpson, E.R. (2001). Estrogen and spermatogenesis. **Endocrine. Rev**. **22**: 289-318.

Oyeyemi, M.O. Olaifa, A.K. and Onwuka, S.K. (2000). Bilateral orchidectomy in West African dwarf goats. Effect on blood electrolyte levels. **Afr. J. Biomed. Res**. **3**: 105-108.

Pagnini-Hill, A. Dworsky, R. and Krauss, R. (1996). Hormone replacement therapy, hormone level and lipoprotein cholesterol concentration in elderly women . **Am. J. Obstet .Gynecol. 174**:897-902.

Parker, T.J. and Haswell, W.A. (1982), Text book the of zoology, 7 ed. Macmillan Press Ltd. London and Basingstoke.p684.

Payne, A.H. Perkins, L.M. Georgiou, M. and Quinn, P.G.(1987). Intra testicular site of aromatase activity and possible function of testicular estradiol. **Steroids**, **50**:435-448.

Pelletier, G. (2002). Effects of Estradiol on Prostate Epithelial Cells in the Castrated Rat. **J. Histochem. Cytochem, 50**: 1517-1523.

Pérez, R.L. Machiavelli, G.A. Romano, M.I. and Burdman, J.A. (1986) Prolactin release, oestrogens and proliferation of prolactin-secreting cells in the anterior pituitary gland of adult male rats. **J. Endocrinol, 108**:399–403.

Prins, G. S. Birch, L., Couse, J. F., Choi, I., Katzenellenbogen, B. and Korach, K. S. (2001) Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor alpha: studies with alpha ERKO and beta ERKO mice. **Cancer Res**.**61**:6089-6097.

Prins, G.S. and Korach, K.S. (2008). The role of estrogens and estrogen receptors in normal prostate growth and disease. **Steroids**, **73**:233–244.

Pylkkanen, L. Makela, S. Valve, E. Harkinen, P. Toikkanen, S. Santti, R. (1993). Prostatic dysplasia associated with increased expression of c-myc in neonatally estrogenized mice. **J. Urol. 149**:1593-1601.

Reece W.O (2005) Dukes, physiology of domestic animals 12th ed. Panima publishing Co. New Delhi/Bangalore. pp

Rhodes, L. Ding, V.D.H. Kemp, R.K. Khan, M.S. Nakhla, A. M. Pikounis, B. Rosner, W. Saunders, H.M. Feeney, W.P. (2000). Estradiol causes a dose-dependent stimulation of prostate growth in castrated beagle dogs. **Prostate, 44**: 8-18.

Richter, C. A. Birnbaum, L. S. Farabollini, F. Newbold, R. R. Rubin, B. S. Talsness, C. E. Vandenbergh, J. G. Walser-Kuntz, D. R. and vom Saal, F. S. (2007) In vivo effects of bisphenol A in laboratory rodent studies. **Reprod. Toxicol, 24** (2):199-224.

Risbridger ,G. Wang, H. Young, P. Kurita, T. Wang, Y.Z. Lubahn, D. Gustafsson, J.A. and Cunha, G.(2001). Evidence that epithelial and mesenchymal estrogen receptor- alpha mediates effects of estrogen on prostate epithelium. **Dev. Biol. 229**:432–442.

Ritchie, J.M. Vial, S.L. Fuortes, L.J. Guo, H. Reedy, V.E. Smith, E.M.(2003). Organochlorines and risk of prostate cancer. **J Occup Environ Med. 45**:692-702.

Roberts, R.O. Jacobson, D.J. Rhodes, T. Klee, G.G. Leiber, M.M. and Jacobsen, S.J. (2004).Serum sex hormones and measures of benign prostatic hyperplasia. **Prostate**, **61**:124–131.

Rommerts, F.F. and Brinkman, A.O. (1981) Modulation of steroidogenic activities in testis Leydig cells . **Mol. Cell. Endocrinol. 21**:15-28.

Rommerts, F.F. de Jong, F.H. Brinkmann, A.O. and van der Molen, H.J. (1982). Development and cellular localization of rat testicular aromatase activity, **J. Reprod. Fertil**, **65**:281-288.

Rowen ,D. Frandson,W. Lee Wilke, and Anna Dee Fails (2009). Anatomy and Physiology of Farm Animals 7th ed pp 409-4010.

Rozati ,R. Reddy, P.P. Reddanna, P. Mujtaba, R. (2002). Role of environmental estrogens in the deterioration of male factor fertility. **Fertil Steril. 78**:1187-1194.

Rubin, M. M. (2007) Antenatal exposure to DES: lessons learned future concerns. **Obstet Gynecol Surv**. **62**: 548-555.

Sanni A. A., Arowolo R. O. A. and Olayemi F. O.(2012). Preliminary study on the effect of castration and testosterone replacement on testosterone level in the New Zealand male rabbit. **Afr. J.Biotechnol**, **11**: 10146-10148.

Schoultz, B.V. Carlstorm, K. Collste, L. Eriksson, A. Henriksson, P. Pousette, A. and Stege, R. (1989). Estrogen therapy and liver function—metabolic effects of oral and parenteral administration. **Prostate, 14** : 389-395.

Searcy, R.L. Reardon, J.E. Foreman, J.A. (1967). A new photometric method for serum urea nitrogen determination. **Amer. J. Med .Technol, 33**:15-20.

Seegers, J.C. van Aswegen, C.H, Nieuwoudt, B.L. Joubert, W.S. (1991). Morphological effects of the catecholestrogens on cells of the

seminiferous tubules of Sprague-Dawley rats. **Andrologia**, **23**:339-345.

Setchell, B.P. and Cox, J.E. (1982) Secretion of free and conjugated steroids by the horse testis into lymph and venous blood. **J. Reprod. Fertil. Suppl**, **32**:123-127.

Setchell, B.P. Laurie, M.S. Flint, A.P. and Heap, R.B. (1983). Transport of free and conjugated steroids from the boar testis in lymph, venous blood and rete testis fluid. **Endocrinol**, **96**:127-136.

Sharpe RM. (1994). Regulation of spermatogenesis. In: Knobil E, Neill JD, editors. The physiology of reproduction. 2end Ed,New York: Raven Press. p. 1363–1424.

Sharpe, R.M. (2003). The 'oestrogen hypothesis'- where do we stand now? **Int J Androl, 26**:2-15.

Siiteri, P.K. and Wilson, J.D. (1970). Dihydrotestosterone in prostatic hypertrophy. The formation and content of dihydrotestosterone in the hypertrophic prostate of man. **J .Clin. Invest**. **49**:1737–1745.

Simoni, R.D. Hill, R.L. and Vaughan,M. (2002). The discovery of estrone, estriol, and estradiol and the biochemical study of reproduction. The work of Edward Adelbert Doisy. **J. Biol. Chem**. **277**: 17.

Simpson, E.R. Mahendroo, M.S. Means, G.D. Kilgore, M.W. Hinshelwood, M.M. Graham-Lorence, S. Amarneh,B. Ito,Y. Fisher, C.R. Michael, M.D. Medelson, C.R. and Bulun, S.E. (1994). Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis, **Endocrin. Rev**. **15**:342-355.

Sisson, S. (1962) .The Anatomy of Domestic Animals. Asian Students ed. 603, Charles E. Tuttle Company, Japan.

Smith, R. and Sizto, R. (1983) metabolic effects of two triphasic formulation containing ethinyl Estradiol and norgesetrel. **Contraception** .**28**:189-199.

Staples, C. A. Naylor, C. G. Williams, J. B. and Gledhill, W. E. (2001) Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates . **Environ. Toxicol .Chem 20**: 2450-2455.

Stevenson, J.C. Rioux, J.E. Koar, L. and Gelfand, M .(2005). 1-2 mg 17β - estradiol combined with sequential dehydrogestrone have similar effects on the serum lipid profile of postmenpasual women: **Climacteric**. **8**:352-359.

Stock, J. Coderre, J. and Mallette, L. (1985): Effect of short course of estrogen on mineral metabolism in postmenopausal women **.J. Clin. Endo. Metab,61** :595-600.

Sutter-Dub, M.T. (2002). Rapid non-genomic and genomic responses to progestogens, estrogens, and glucocorticoids in the endocrine pancreatic-cells, the adipocyte and other cell types. **Steroids**.**67**:77-93.

Tabcco, A. Meiattini, F. Moda, E. and Tarli, P.(1979). Simplified enzymic/colorimetric serum urea nitrogen determination. **Clin. Chem 25**:336-337.

Tam, N.N. Szeto, C.Y. Freudenberg, J.M. Fullenkamp, A.N. Medvedovic, M. Ho, S.M. (2010). Research resource: estrogen-driven prolactin-mediated gene-expression networks in hormone-induced prostatic intraepithelial neoplasia. **Mol. Endocrinol, 24**: 2207-2217.

Todiodi, M. (2010). The effects of exogenous estrogens on estrogen receptors in male reproductive organs. Interdisciplinary .**J. Health. Scie, 1**: 73-82.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with and alternative oxygen acceptor. **Ann. Clin. Biochem, 6**:24-27.

Tsai, C.L. and Wang, L.H. (2000). Sex differences in the responses of serum calcium concentrations to temperature and estrogen in Tilapia, *Oreochromis mossambicus*. **Zool. Stud, 39**: 55-60 .

Tsai-Morris, C.H. Knox, G. Luna, S. Dufau, M.L. (1985) Acquisition of estradiol-mediated regulatory mechanism of steroidogenesis in cultured fetal rat Leydig cells. **J. Biol. Chem, 261**:3471-3474.

Van Coppenolle, F. Le Bourhis, X. Carpentier, F. Delaby, G. Cousse, H. Raynaud, J.P. Dupouy, J.P. Prevarskaya, N. (2000) Pharmacological effects of the lipidosterolic extract of Serenoa repens (Permixon) on rat prostate hyperplasia induced by hyperprolactinaemia: comparison with finasteride. **Prostate 43**: 49–58.

Van Coppenolle, F. Skryma, R. Ouadid-Ahidouch, H. Slomianny, C. Roudbaraki, M. Delcourt, P. Dewailly, E. Humez, S. Crépin, A.

Gourdou, I. Djiane, J. Bonnal, J.L. Mauroy, B. Prevarskaya, N.(2004). Prolactin stimulates cell proliferation through a long form of prolactin receptor and K+ channel activation. **Biochem. J, 377**: 569-578.

Van Coppenolle, F. Slomianny, C. Carpentier, F. Le Bourhis, X. Ahidouch, A. Croix, D. Legrand, G. Dewailly, E. Fournier, S. Cousse, H. Authie, D. Raynaud, J.P. Beauvillain, J.C. Dupouy, J.P. Prevarskaya, N. (2001). Effects of hyperprolactinemia on rat prostate growth: evidence of androgen-dependence. **Am. J. Physiol. Endocrinol. Metab, 280**: 120–129.

van der Molen, H.J. Brinkmann, A.O. de Jong, F.H. and Rommerts, F.F.(1981). Testicular oestrogens. **Endocrinol** . **89**:33-46.

Verma, S. Srivvastava, R.K. Sood, S. and Sharma, S.(2005). Effect of estrogen on hypoglycemia- induce neurogilial impairment in overectomized rats. **Methods. Find. Exp .Clin .Pharmacol** .**27**:405 -409.

Vom Saal, F. S. Cooke, P. S. Buchanan, D. L. Palanza, P. Thayer, K. A. Nagel, S. C. Parmigiani, S. and Welshons, W. V. (1998) A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. **Toxicol. Ind. Health**,**14**: 239-260.

Waites, G.M. and Einer-Jensen, N. (1974). Collection and analysis of rete testis fluid from macaque monkeys. **J. Reprod. Fertil**, **41**:505-508.

Walsh, P.C. Hutchins, G.M. and Ewing, L.L. (1983). Tissue content of dihydrotosterone in human prostatic hyperplasia is not supranormal. **Clin. Invest, 72**:1772–1777.

Wang, T.J., Slawin, K.M., Rittenhouse, H.G., Millar, L.S. and Mikolajczyk, S.D (2000). Benign prostatic hyperplasia – associated prostate-specific antigen (BPSA) shows unique immune reactivity with anti-PSA monoclonal antibodies. **J. Biochem, 267**: 4040-4045.

Ware,J.L.(1994). Prostate cancer progression implication of histopathology. **Am. J.Pathol, 154**: 983-993.

Wellerson, R.S. Daniel, E.S. Silvana, G.P. Campos, L.S.C. Patricia, S.L.V. Sebastiao, R.T. (2008). Oestrogen supplementation following castration promotes stromal remodelling and histopathological alterations in the Mongolian gerbil ventral prostate. **Int. J. Exp Pathol**, **89**: 25-37.

Wells, L.J. (1935) Seasonal sexual rhythm and its experimental modification in the male of the thirteen lined ground squirrel. **Anat. Rec**. **62**: 409-444.

Welshons, W. V. Nagel, S. C. and vom Saal, F. S. (2006) Large effects from small exposures. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. **Endocrinolo** .**147**:56-69.

Weniger, J.P. Chouraqui, J. and Zeis, A. (1993). Production of estradiol by the fetal rat testis. **Reprod. Nutr. Dev**. **33**: 121-127.

Williams, K. McKinnell, C. Saunders, P. T. Walker, M. Fisher, J. S. Turner, K.J. Atanassova, N. and Sharpe, M. (2001) Neonatal exposure to potent and environmental oestrogens and abnormalities of the male reproductive system in the rat: evidence for importance of the androgen-oestrogen balance and assessment of the relevance to man. **Hum Reprod Update**, **7**: 236-247.

Woo, N.Y.S, Chung A.S.B, and Ng, T.B (1993). Influence of oral administration of estradiol-17β and testosterone on growth, digestion, food

conversion and metabolism in the under yearling red sea bream, *Chrysophrys major*. **Fish. Physiol .Biochem. 10**:377-387

Xing-Jiu, H . Yang-Kyu, C. Hyung-Soon, I. Oktay, Y . Euisik, Y. and Hak-Sung, K. (2006). Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. **Sensors** 6: 756-782.

APIENDEX

A1

Dissection of the male rabbits and collection of prostate tissue

THE PUBLISHED PAPERS