American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Estradiol-Induced Testicular Toxic Alterations in Wister Rats During Adolescence: Anatomical and Histological Investigation

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Received: 2025, 15, Nov
Accepted: 2025, 21, Dec
Published: 2026, 31, Jan

Annotation: The present investigation was conducted to characterize the structural susceptibility of the male gonadal tissue to exogenous estrogen exposure during the pubertal window, with particular emphasis on morphological regression and histological remodeling induced by estradiol benzoate administration. Methods: twenty male rats at two months aged were split equally into two groups:(control, and treated). The treatment rats were given sub\cutaneous injections of 60 µg/ of rat oestradiol benzoate dissolved in 0.26 ml of olive oil for 15 consecutive days; while control rats were given subcutaneous injections of 0.26 ml of olive oil. Twenty-four hours following final treatment dose, Rats in both groups were given general anesthesia and weighed., had their scrotums dissected and had their testicles separated from other organs. Weight, length, width, and volume calculated. For histological examination, sample from testis were kept in 10% natural formalin. Using hematoxylin and eosin (H&E) staining, identify general architecture and histopathological changes. A unique stain called Masson's trichrome staining is used to detect collagen fibres. The results showing the testicular morphometric

measurements in control rats were similarly to intact; while all data showed a marked decrease in treatment rats (G2). Histological analyses showed that control rat testis in with full structural and histological integrity; while estradiol treated rats testis presenting significantly decreased in diameter and epithelial height of seminiferous tubules ($p \leq 0.05$) and there were histological alterations, including irregular, atrophy of seminiferous tubules, and loss of organized stratified epithelium. There was no evidence of typical Leydig cells. This research determined that observed changes clearly demonstrate how estradiol impacted the histological makeup and reproductive capabilities of testis, leading to reduced spermatogenesis and diminished secretory function of interstitial cells. Consequently, overall findings affirm rats subjected 60 µg of estradiol benzoate each rat. during their puberty stage exhibited toxic effecting on testis, ultimately resulting in decreased male fertility.

Keywords: testis toxicity, Estradiol benzoate, Puberty, infertility, Wister rat.

Introduction

Reproductive structures are generally alike across most species, consisting primarily of the testes, spermatic ducts, and accessory sex glands. The generation of steroid hormones (steroidogenesis) and spermatozoa is carried out by the testes, two organs with exocrine and endocrine functions. which are created by a complicated and well-coordinated process called spermatogenesis, these Production is The testicular somatic cells and germ cells must develop normally. (Salah and Abdul-Hamid ,2014; M¨akel¨a et al.,2019). This event appears to be regulated by oestrogens, which operate through nuclear oestrogen receptors (ESRs) ESR1 and ESR2, however it is also influenced by many paracrine and endocrine factors (Bois et al., 2010)

The male reproductive system's growth and operation are closely connected to intricate hormonal interactions, with the pulsatile hormone release from the HPG axis being crucial for male fertility. Timely diagnosis and treatment are crucial for managing hormonal disorders that can affect reproductive health and overall well-being (Li et al., 2024)

The inability to conceive naturally following a year of consistent sexual activity without the use of contraception is known as infertility. It is frequently challenging to pinpoint the exact cause of male infertility, which could be of primary or acquired origin, copulatory or fertilization issues, or anything else that could impair male reproductive capacity. (Prochowska **and Niżański**.,2022). The causes of male infertility are diverse and include hormonal imbalances, genetic defects, structural disorders of the reproductive system, as well as psychological and behavioral factors, in addition to adverse environmental influences (Serdarogullari, 2020; Agarwal et al., 2021). Infertility is estimated that approximately 15% of couples experience

infertility (Vander Borght & Wyns, 2018). Male-related variables are responsible for almost half of infertility cases. (Minhas et al., 2021; Rowaiee et al., 2025), while idiopathic male infertility accounts for 15–30% of all reported cases (Kawwass & Dokras, 2008).

Conventionally, testosterone has been viewed as the male sex hormone and estrogen as the female sex hormone. Estradiol, the main type of estrogen, is also essential for male sexual function (**Walker and Cooke 2023**) For male libido, erectile function, and spermatogenesis, estradiol is essential. The brain, penis, and testis—all essential organs for sexual function—are rich in estrogen receptors and the enzyme aromatase, which converts testosterone to estrogen. In areas of the brain linked to sexual excitement, estradiol synthesis is increased. Additionally, the penis has a high density of estrogen receptors close to neurovascular bundles, which are dispersed throughout the corpus cavernous. Erectile dysfunction is independently increased by decreased testosterone and increased estrogen. From the hypothalamus-pituitary-gonadal axis to Leydig, Sertoli, and germ cells to the ductal epithelium, epididymis, and mature sperm, estrogen affects spermatogenesis in the testes at every stage. Estradiol regulates testicular cells in a way that is both stimulatory and inhibitory, demonstrating a complicated interaction between dose-dependent and time-sensitive modulation. (**Schulster** et al.,2016).

Numerous physiological processes, like as cardiovascular function, ovarian endocrine and reproductive function, and even male reproductive function, have been shown to be impacted by estrogen. (Pan et al., 2024). Due to the varying distribution of aromatase (P450arom) in the testes of different species, the site of estrogen synthesis in males has been thoroughly investigated during the past ten years. In the male reproductive system, at least three distinct testicular cell types—Leydig, Sertoli, and germ cells—as well as the epithelial cells of the epididymis produce estrogens. Sertoli cells are the main source of estrogen in the juvenile testis, whereas Leydig cells and germ cells express P450arom in the adult testis. There is currently mounting evidence that the primary source of estrogens in the male reproductive system is germ cells. (Bilińska et al., 2006)

Similar to other steroid hormones, the action of estrogen is mediated by certain intracellular receptors in target cells. In the testis, both estrogen receptor beta (ERβ) and alpha (ERα) are expressed, but their expression profiles differ. While ERβ is present in germ cells, Sertoli cells, and fetal Leydig cells, ERα is discovered in the nucleus of the Leydig cells in mouse testes. (Nilsson, and Gustafsson, 2002)

Estrogen plays a complex regulatory role in multiple tissues and organs of the body, with closely associated with the maintenance of reproductive health (**Zhang et al., 2023**). Its effects begin in the early embryonic stage, where excess estrogen can lead to alterations in the development of some reproductive structures. It also contributes to the regulation of male fertility, also with rodent studies showing that the absence of estrogen receptor alpha or the aromatase enzyme is associated with infertility. The regulation of male fertility in humans is more complex, and the precise functions of estrogen are still not fully understood (Cook et al., 2021).

It has long been known that the development and maturation of secondary sexual traits depend heavily on sex steroid hormones. However, since then, there has been a significant shift in our knowledge of the biology of sex hormones, and it is now believed that progesterone, oestrogen, and androgens are crucial for a number of physiological and pathological processes in humans **(Nilsson, 2023**)

Due to increased developmental exposure to endocrine-disrupting chemicals (EDCs), men's reproductive health is worsening in the modern world (Kaushik et al., 2023; Blazhkova, 2025). The USA Environmental Protection Agency (EPA) formally defined endocrine disrupting chemicals (EDCs), formerly known as xenoestrogens, as exogenous substances that interfere with the natural processes of hormone synthesis, secretion, transport, binding, action, or removal. (Diamanti-Kandarakis et al., 2009, Yilmaz et al.,2020; Plante et al., 2022; Tzouma et al.,2025). The hypothalamic-pituitary-gonadal (HPG) axis regulates sex hormone biosynthesis, and it is

well recognized that EDCs can interfere with normal gonad function (Czarnywojtek et al., 2023, Lei et al., 2023). Spermatogenesis and steroidogenesis, as well as follicle growth, development, maturation, and ovulation, are all influenced by the HPG axis, which is a major modulator of reproduction. (**Xie et al., 2022, Koysombat et al., 2023**). EDCs have the ability to pass through the blood–brain barrier, which may lead to hypothalamus inflammation and interfere with the reproductive axis (Stathori et al., 2024). These substances may have androgenic, anti-androgenic, estrogenic, or anti-estrogenic properties, all of which are essential for preserving development, behavior, reproductive processes, and homeostasis (Akingbemi et al., 2004; Lukacova et al., 2012; Pan et al., 2024). there for numerous studies were started using animal models that were given estrogen or estrogen-like substances during fetal or neonatal life and they revealed to a variety of inimical effects of exoestrogen on parenchyma of testis and the impacts that followed in adult animals also were noted (Oishi, 2002; Akingbemi et al., 2004). Delbes et al., (2010) who reported there is increasing evidence linking exposure to environmental estrogen and endocrine disruptors to a decrease in human fertility as well as an increased risk of testicular cancer and raise the possibility of reproductive toxicity, which has a negative impact on fertility. (Srilatha and Adaikan, 2011; Chen *et al*., 2020). Human evolution will now take place in a complicated chemical terrain of our own creation. It is clearly obvious that contact with EDCs is already negatively impacting reproductive biology and conduct in ourselves and our planetary counterparts and will keep doing so for future generations (Patisaul, 2021). The purpose from this study evaluates the effect of 60 µg of estradiol on the testis of rats and for any possible toxicological effects on male reproductive system.

Materials and methods

Experimental animals

Twenty male Wister rats, approximately two month of age (pubertal), weighing nearly 200 gmwere applied in this investigation. The animals were kept at the University of Al-Qadisiyah's College of Veterinary Medicine in normal metal cages with five rats each. under controlled housing conditions that included a controlled temperature (21–25°C), 55–60% relative humidity, lighting cycle of 14 h light/10 h dark and adequate ventilation. The rats were provided with a balanced soy-free diet and drinking water ad libitum. A 14-day acclimatization period was completed before the experimental procedures began. All steps related to animal husbandry and handling were carried out in accordance with the approved standard guidelines for the care and use of animals in scientific experiments. Only healthy rats were employed in this study to avoid any physiological or biological changes to the rats' organs and tissue. Prior to and following the therapy, the rats in both groups were weighed.

Chemical Substance:

The supplier of estradiol benzoate was Sigma-Aldrich Co. in St. Louis, Missouri, USA. 0.26 cc of olive oil (vehicle) was used to dissolve 60 micrograms of estradiol.

Experimental Design

Twenty male Two primary groups of ten Wistar rats each were randomly assigned. Animals in the control group received a subcutaneous injection of 0.26 cc of olive oil. daily for 15 consecutive days. Five rats were used for anatomical studies and five for histological examination. **Estradiol-treated group**: Animals were subcutaneously injected with 60 micrograms of estradiol per day for fifteen days straight, dissolved in 0.26 milliliters of olive oil.Five rats were used for anatomical studies and five for histolopathlogical examination (Goyal et al., 2003; Kaushik et al., 2010)

Body Weight Measurement and Euthanasia Procedures

The weights of male rats in all experimental groups were measured both before to and following the initiation of treatment period using a highly sensitive, high-precision balance. One day after

the end of the treatment period, animals in both groups were euthanized by an overdose of an anesthetic mixture combination ketamine (80 mg/kg body weight) and xylazine (10 mg/kg body weight) to produce anesthesia.

Anatomical study

Incision (5 cm) was made in the mid-scrotum using a No. 15 surgical blade (Aesculap AG and Co. KG, Tuttlingen, Germany) to access the testes. After careful dissection and removal of surrounding tissue, the right and left testes were removed from each animal. The shape and morphometric of the both testes were recorded.

Parameters evaluated

1- Body weight and morphological observations: On the first day of adaptation, the animals' body weights were noted., and the final day of sacrifice.

2-The relative weights of each of the testis were calculated with the use of the formulae below: Relative Weight (%) = Testis weight (g) ÷ Animal weight (g) ×100 (Adegoke et al., 2025)

3-Testicular dimensions (length, and width) were accurately measured using a high-sensitivity Mituyoto caliper (±0.05 mm) to determine the longitudinal (Craniocaudal axis) (mm) and transverse diameters (Mediolateral axis) (mm) of each testis (Olukole et al. (2009).

4- Testes volume: We picked The most popular approach for measuring things with irregular shapes is the water replacement method. A graduated cylinder was filled with a predetermined volume of water. After that, the graded cylinder was carefully filled with the testis. The testis's volume was computed by deducting the water's initial and final volumes. (**Parhizkar et al., 2014).**

Histological Study

Testes from all groups were washed with normal saline and then After being separated, the testes were preserved in 10% neutral buffered formalin. Testicular slices that were 5 mm thick were sliced and re-fixed in new fixative for an additional 24 hours following first fixation. The fixative testis were dried in increasing alcohol grades (50, 70, 80, 95, and 100%), cleaned in xylene, infiltrated in molten paraffin wax, and then inserted in molten paraffin wax to create blocks as part of standard histological processing. The material-containing paraffin block was subsequently cut at a thickness of 5 µm using a rotary microtome, and the paraffin wax was removed with xylene, and the sections were rehydrated using a descending alcohol series before being stained and histologically examined (Luna, 1968).

Procedure for Staining

For routine histological inspection of paraffin sections, the standard Hematoxylin and Eosin technique (Bancroft and Gamble, 2008) and Masson's trichrome staining (Luna, 1968) are used.

Histometric Measurements

The extent of testicular toxicity was evaluated by measuring the diameter of the seminiferous tubules, and height of epithelium.

Five random fields from each testis were selected for measurement of seminiferous tubule diameter in micrometers (µm) at 20× magnification using Image Pro Plus software connected to an Olympus BX-40 microscope, enabling accurate quantitative data for morphometric measurements. Tubular diameter (TD) was assessed using H&E-stained sections.

The height of the germinal epithelium was measured using the same seminiferous tubules used to measure tubular diameter. The height of the epithelium was considered to extend from the basement membrane to the final stage of germ cells (spermatocytes). Cellular changes within the seminiferous tubules were observed.

Statistical analysis

Statistical analysis of the group differences with regard to all measured data parameters was done using one-way analysis of variance and with version 24 of the SSPS software. $P \leq 0.05$ were regarded as significant in the calculations.

Results

Anatomical results:

The transparent tunica vaginalis and tunica albuginea cover the testicles of the Wister rat in the study's control group (non-treated) that are suspended in the scrotal sac caudo-ventral to the penis. The testis's ellipsoidal form and cream to milky-white color. The testis was attached to the body of the epididymis loosely, connected to the tail of the epididymis by a ligament, and held in place at the cranial pole by the fat-covered head of the epididymis. Numerous blood vessels that are clearly wavy are seen on the outside of the surface (Figure 1A&B). This finding was in line with studies on the gross morphologic appearance of testicles in several rodent species by Olukole et al. (2009); and Omirinde et al. (2021).

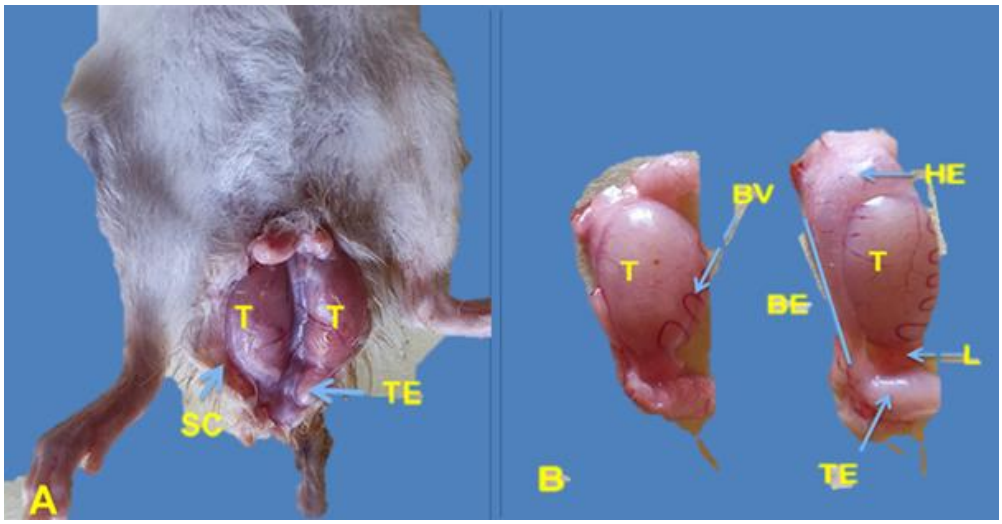

Figure (1): Photograph of testis (T) in control group (non-treated) of Wister rat at age two month. A: showing location of testes inside scrotum sac (SC). B: Showing relation shape of testis with epididymis partitions (head (HE) that covered with fat (F), body (BE) and tail (TE) that connect with testis by ligament (L)). Blood vessels (BV) appears as wave across external surface of testis

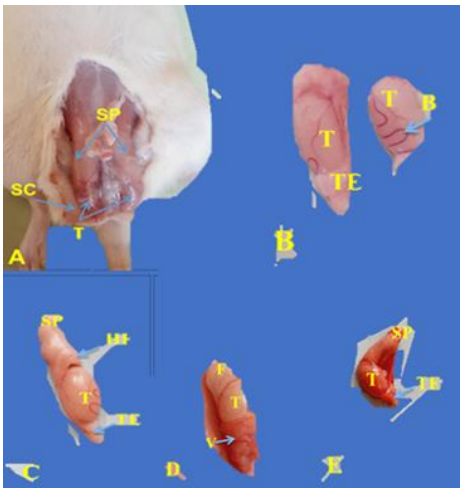

Figure (2): Photograph of testis(T) in treated group of Wister rat at age two month; showing the testis were appeared inconstancy oval to elongated in shape but smaller in size than that in control group. A: showing location of testes inside scrotum sac (SC) and tunica vaginalis

(TV), suspended by spermatic cord (SP). B, showing the testis attached with epididymis partitions (head (HE), body (BE) and tail (TE)). Blood vessels (BV) appear as wave across external surface of testis C, D, and E: showing effect of harmful estradiol on the shape of the testis. **This result conforms to Onuoha**, (2020); and Rajathi et al., (2021).

Table 1: showing the body weight and relative weight of the Wister rat testis at two month aged decrease in treated groups (G2) with 60 µg/ rat of estradiol when compare with control groups (G1)

Measure / Groups	Body weight (BW) (g/ mg/100 g BW	Relative weight of testes (mg/100 g BW)	t value	p value
G1	183±7.81	0.65±0.11(A)	4.31	0.0015
G2	165±6.59	0.39±0.24 (B)	2.41	0.0365

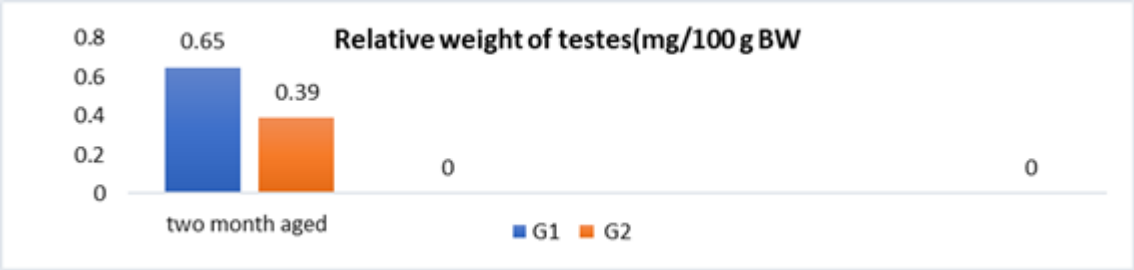

Figure (3): Showing the body weight and relative weight of the Wister rat testis at two month aged decrease in treated groups (G2) with 60 µg/ rat of estradiol. When compare with: control groups (G1)

Table (2) and Figure (4): showing the dimensions of the right and left testes of rats in current study was revealed differences in mean of the length, width and volume between the two groups, the testis of rat in the control group (non-treated group) showed that the mean of the length of the right and left testis were as (18.154±0.275) mm. (18.44±0.585) mm. respectively. The mean width of the right and left testis were as (10.292±0.183) mm. (9.87± 0.088) mm. respectively; while the mean volume of both testes showed that most of the samples were within the range of 1.0–1.1 cm³. Overall, the results showed that the right and left testicles-maintained symmetry and similarity in length, width, and volume, with significant differences at **(P≤0.05%)**, also the weight of both testis of rats was constancy, this indicate that reflecting of the integrity of the anatomical structure and normal reproductive tissue function. This was in line with the findings of Hess & de Franca (2009) who claimed that testicular balance was a crucial sign of tissue stability and reproductive effectiveness; While the present study found that when adult male rats were given a dose of estradiol (60 micrograms/rat); there were noticeable morphometric changes in both testicles. These changes included a more or less uniform appearance, which was reflected in decreased testis weights and volumes, as well as a decline in the longitudinal and transverse dimensions of the testicles when When compared to the non-treated control group, there were notable variations at P≤0.05%. The left testis measured 16.476±0.468 mm, while the right testis was 16.406±0.712 mm. These data indicate a considerable reduction in testicular length as compared to the control group.The mean width of the right and left testis appeared as (8.844± **(0.529mm**. and (7.8± **0.628)** mm. respectfully. Reflecting a decrease in the transverse diameter, particularly in the left testis. The mean weight of the right and left testis appeared as (0.672± **0.083)** gm. And (0.656 ±**0.102**) gm. respectfully. These values are significantly lower than that in control group, this explains by Onuoha, (2020) who consider, due to its strong and positive link with sperm production, testicular weight is a crucial factor in evaluating guys' reproductive potential. The mean of the volume of the right and left testis was recorded and appeared as (0.61±**0.179**) cm^3 and (0.53**±0.164)** cm³. Respectfully, this suggests that testicular volume significantly decreased following oestradiol administration. The majority of the results

showed that the testes in the treated group were smaller than those in the control group in terms of weight, volume, and longitudinal and transverse dimensions. This result conform Kaushik et al., (2010) who report decrease in weights of testis and accessory sex organs due to estrogen injection and The number of growing germ cells per tubule decreased in tandem with the testis weight. According to Alhussein and Albghdady (2024), this may indicate that oestradiol injections change the balance of hormones. As a result, there is less androgen receptor (AR) immunolocalization in the testis, which inhibits the growth and function of the testicles. According to earlier research like Sharpe, (1998); Norazit et al., (2012); and Hess, (2021), oestrogen medication causes testicular atrophy by inhibiting the hypothalamic-pituitary-testicular (HPT) axis, which lowers luteinizing hormone (LH) release and hinders the activation of Leydig cells to make testosterone **.**Additionally, the sex-specific organization of many neuroendocrine pathways depends on the gonadal hormones; hence, endocrine disruption defects in these pathways might result in permanent harm to the testis (Cooke et al., 2021; Heather and Patisaul, 2021; Corpuz-Hilsabeck and Culty, 2023).

The results of the statistical analysis showed a significant decrease in all morphometric measurements (length, width, weight, and volume) in the estradiol-treated group (G2) compared to the control group (G1), indicating an inhibitory effect of estradiol hormone on the growth and development of the testes in male rats.

Table 2: Morphometric of both testis of Wister rat at two month age in control group (non-treated) (G1) and treated group with estradiol 60 micrograms /rat (G2) (Length, Width, weight and Volume)

measure	Length (mm)		Width (mm)		weight (gm)		Volume (cm^3)	
	Right	Left	Right	Left	Left	Right	Left	Right
G1	18.154 ±0.275	18.442 ±0.585	10.292 ±0.183	9.87 ±0.088	1.198 ±0.026	1.226± 0.01	1.02 ±0.02	1.04 ±0.02
G2	**16.406 ±0.712**	**16.476 ±0.468**	**8.844 ±0.529**	**7.826 ±0.628**	**0.656 ±0.102**	**0.672 ±0.083**	**0.53 ±0.164**	**0.61 ±0.179**
T - test	**2.29**	**2.62**	**2.59**	**3.22**	**5.15**	**6.63**	**2.97**	**2.39**
P value	**0.045**	**0.025**	**0.027**	**0.009**	**0.001**	**0.001**	**0.014**	**0.038**
	Significant p < 0.05	**Significant p < 0.05**	**Significant p < 0.05**	**High Significant p < 0.001**	**High Significant p < 0.001**	**High Significant p < 0.001**	**High Significant p < 0.001**	**High Significant p < 0.001**

In comparison to the control group (G1), the statistical analysis revealed a significant decrease in all morphometric measurements (length, width, weight, and volume) in the estradiol-treated group (G2). This suggests that the hormone estradiol inhibits the growth and development of the testes in male rats.

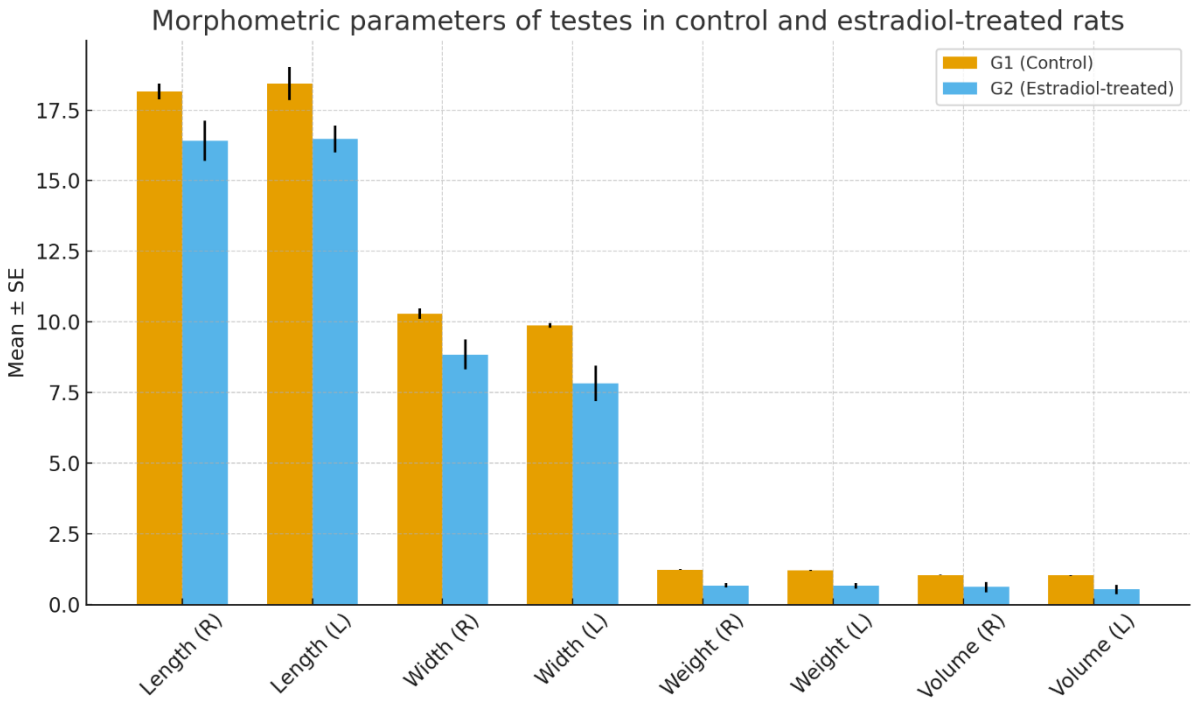

Figure (4) clearly shows difference between control group (G1) and the estradiol-treated group (G2) in all morphometric measurements (length, width, weight, volume) of the testis of Wister rat at age two month. A significant decrease was observed in all values in the treated group (G2), confirming the inhibitory effect of estradiol hormone on testicular growth and development in male rats.

Histological results of the Wister rat testis: Control group (non-treated))

Histological examinations in the control group (non-treated) showed that the testicular parenchyma reserved their intact histological structure. The seminiferous tubules appeared regular nearly round in shape and contained all germ cells at successive stages of spermatogenesis through primary and secondary spermatocytes, to spermatids and mature sperm directed toward the lumen of the tubule. The seminiferous tubules basement membrane appeared intact and, homogeneous and surrounded by smooth muscles. Sertoli cells (polyhedral in shape) were clearly visible within the spermatogenic epithelium, that playing a supporting role in maintaining the microenvironment of the tubules. Each seminiferous tubule was surrounded by a thin interstitial tissue (loose connective tissue). Contained Leydig cells (Figure 5). These results conform to **Hussein et al., (2020)**. The present of Leydig cells reflecting their secretory activity in testosterone production. These effectiveness of the reproductive process and the integrity of the testicular microenvironment similar observations were also Stan (2015) found similar results in adult guinea pigs.; Androma and Khasanah (2017) and El-Azab and El-Mahalaway (2019). Rajathi et al., (2021) who found Sertoli cells are big, oval-shaped cells with an uneven, faintly pigmented nucleus.. This may be due to species difference.

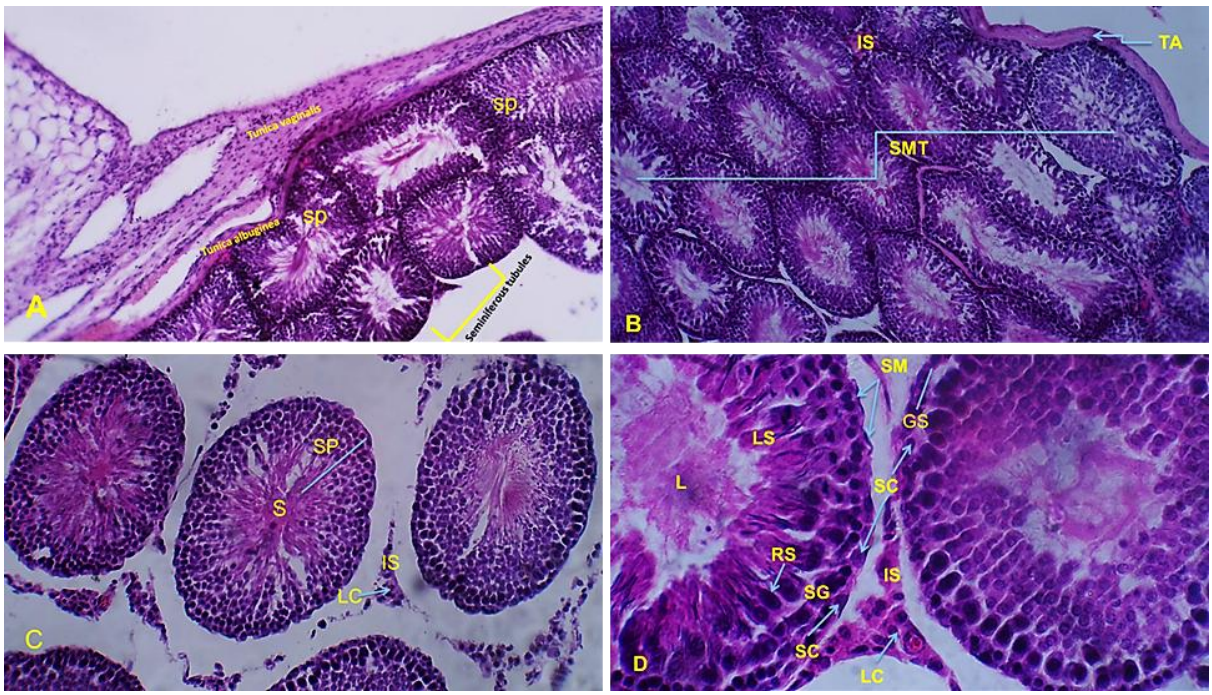

Figure (5): Microscopic section of testis of Wistar rats in control group (non-treated) showed covered by tunica vaginalis and albuginea (TA)and somniferous tubular (SMT) that rest on basement membrane that surrounded by smooth muscles (SM)and lined with pseudostratified epithelial that consist of step of spermatogenesis (SP) ((primary sparmatogonia (SG), and round spermatid (RS, longitudinal spermatid (LS))) between them Sertoli cells (SC), lumen of tubules(L) full with sperm (S). interstitial tissue (IS) have Leydig cell (LC). H&E stain; A and B = 100X, C= 200X D= 400X.

Histological results of the Wister rat testis in treated group

In the present study, the microscopic sections of the rats testis from the estradiol-treated group revealed clear histopathological changes occurred when compared with histological structures of the testis in the control group (non-treated).The histopathological changes included small diameters with atrophy or shrinkage of the seminiferous tubules which appeared have deformed and exhibited varying degrees of that irregularly shaped Figure 6 A and B) , and the tubular epithelium was primarily vacuolated, withered, loss of the normal stratified structure(disorganization) of the spermatogenic epithelium with showing most of the epithelial germ cells had lost their morphologies like prevalence germ cell hypoplasia and decreased epithelium density. Elongated spermatids and sperms were wholly lacking, these results reflecting a halt in spermatogenesis (Figure 6 C and D) , this result conform with D'Souza et al.,(2005) ; and Kaushik *et al.,* (2023) who found that 65% of rats treated with diethylstilbestrol (DES) had testicular tumors, fewer seminiferous tubules in Stage VIII, a lower sperm count, and infertility as a result of prenatal endocrine disturbance. and with Gill-Sharma et al. (2001) who establish when adult male rats treated with 17β-estradiol at a level of 100–1,000 µg/kg/day for 60 days experienced total azoospermia. This pattern of testicular injury is had been established by several investigators like Chaki et al., (2006); Mohamed and Arafa, 2012; Ullah et al., (2019); and **Abarikwu et al., (2023**). Some seminiferous tubules containing only Sertoli cells or primary germ cells. The wide interstitial spaces were observed and a marked showed signs of atrophy and decrease in the number or absence of typical Leydig cells or hypoplasia of Leydig cells (Figure 6 B and C). Akingbemi (2005), demonstrated that chronic exposure to estrogens leads to an imbalance in sex hormones, inhibiting germ cell divisions and negatively affecting Leydig cell activity, thereby impairing testosterone production and this observation agreements with these reports by Norazit et al., (2012) whom conclude that exposure to 17ß-estradiol during puberty stops the seminiferous tubules maturation and with (Della Seta et al., 2006; **Adeoye et al.,**

(2017).

The present research identified vacuolations within the epithelial tubules. This outcome could be attributed to heightened fatty acid utilization resulting from elevated estrogen levels. Parrish et al. (1999) proposed that oxidative stress affects cadherin/catenin complexes, leading to impaired cell-to-cell adhesion and membrane integrity due to oxidative stress caused by estradiol. Jensen et al. (1994), Herrero et al. (2005), and Raut et al. (2021) suggested that estrogen aids in the elimination of free fatty acids (FFA). Dutta et al.,(2021); Stathori et al. (2024) proposed that contact with endocrine-disrupting chemicals (EDC) is associated with neuro-inflammation, particularly affecting the hypothalamic regions of the gonadotropic axis, which may influence the impact of EDC exposure on reproductive dysfunction . Aitken and Roman (2009) explained that in adult rats, treating with any chemicals that lower the intra-testicular concentration of testosterone. Testicular injury observed by (Takahashi and Oishi, 2006) and testicular morphology changes and the loss of spermatogenic cells at all stages of the spermatogenic cycle have been observed in rats exposed to chronic and intermittent hypobaric hypoxia; as a result, oxidative stress in spermatogenic cells(Zepeda et al.,2014).

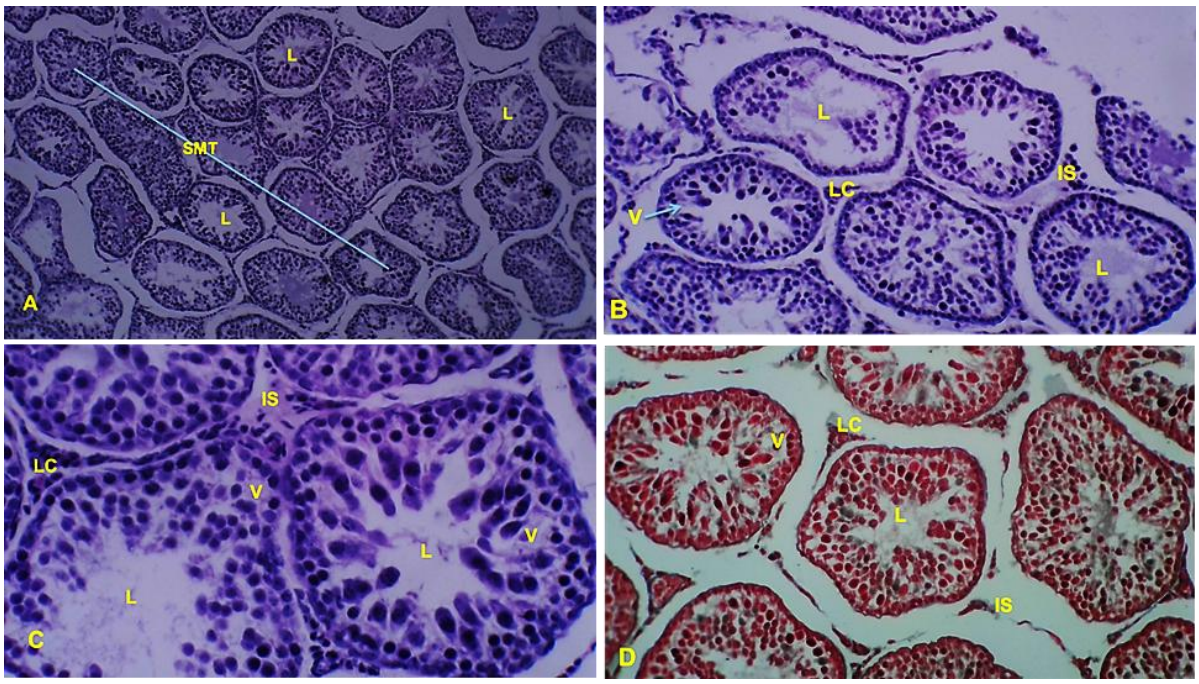

Figure (6): Microscopic section of testis of Wistar rats in treated group with 60µg/rat of estradiol benzoate, showed irregular shape of somniferous tubular(SMT) and atrophy that rest on irregular basement membrane , showed most tubular lumen(L) were irregular in shape, shrinking, smaller, disorganization) of the spermatogenic epithelium and decrease in number of sperms or absent, vacuolations within the epithelia tubules (V) interstitial tissue (IS)between tubule degeneration had few Leydig cell (LC). H&E stain; A =100X; B = 200X, C= 400X. Masson's trichrome staining; D= 200X.

Histological results of the measurement of the seminiferous tubules.

The epithelial layer of the seminiferous tubules of Wister rat testis in the control group showed consistent values with no significant differences, confirming the integrity of the cellular structure and the preservation of the normal organization of the seminiferous epithelium without of the histopathological changes. The result showing the mean height of the epithelial of the seminiferous tubules was recorded as (178.91 ± 6.71) µm. (Table 3 and Figure 7). These observations are consistent with Russell et al. (1990) and Johnson (2014), who stated that the regularity of germ cell rows , Sertoli and Leydig cell were indicators of efficient spermatogenesis and result of the hormonal balance ; while the results of histological measurements of the epithelial layer of seminiferous tubules of the rat testis in the treated group showed the mean

height of the epithelial layer was (52.21 ± 4.99) µm. with a significant decrease compared to that in the control group. These results reflecting a direct pathological effect on the testis tissue and structural integrity of the epithelial cells (Table. 3 and Figure 7)

The findings of the present investigation reveal that a dosage of estradiol (60 micrograms/rat) administered to adult male rats caused distinct histopathological alterations in the micrometric structure of testicular tissue, including a reduction in cell mass within seminiferous tubules due to diminished spermatogenesis and atrophy of interstitial tissue, as

Evidenced by research conducted by Sharpe (2010); Johnson (2014); and Mahboob et al. (2025), leading to heightened susceptibility of the seminiferous epithelium to both environmental and therapeutic influences. This aligns with findings from other research that has shown estrogen exposure results in germ cell degeneration (Akingbemi, 2005; Atanassova et al., 2005). These findings clearly illustrate a direct connection between hormonal equilibrium and the structures and functions of the testis, as alterations may indicate reduced cell division or partial atrophy of testicular tissue, consistent with Leavy et al. (2017), who show that elevated estradiol levels significantly affect the human testis, and with Tirpák et al., (2021); and Zhao et al., (2025) who demonstrated in their study of the effects of certain exogenous compounds on testicular tissue. Defects in undifferentiated spermatogonia can result in testicular germ cell tumours or sub- or sterility (O'Donnell et al., 2021).

Table. 3. Histometric measurement of Height of epithelial layer seminiferous tubules (µm) of the testis of Wister rat at age two month in control group (non- treated)(G1) and treated group with estradiol 60 micrograms /rat (G2)

Group	Height of epithelial layer seminiferous tubules (µm)
G1	78.91 ±6.71
G2	52.209 ±4.99
T – test	7.82
P value	1.43
There is a significant decrease in height of epithelial layer seminiferous tubules (µm)in the treated group (G2) compared to the control group (G1)	

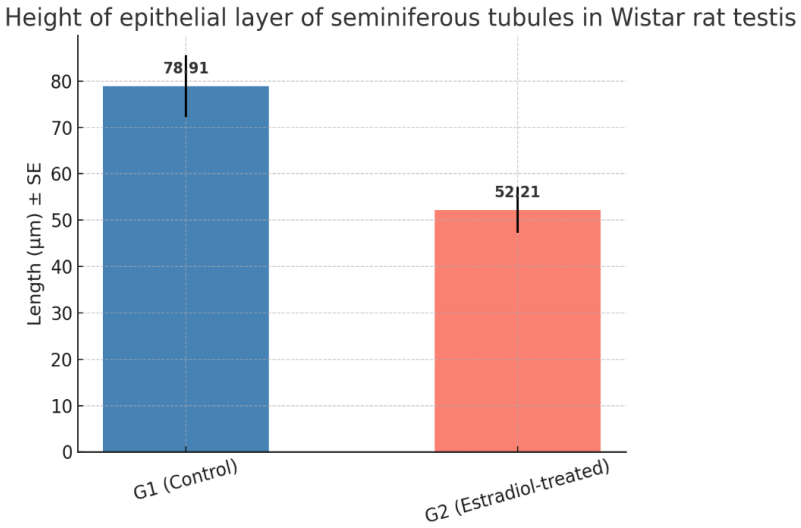

Figure (7): Shows a significant decrease in the height of the seminiferous tubule epithelium (µm) of the testis of Wister rat at age two month in the estradiol-treated group (G2) compared to the control group (G1), reflecting the strong inhibitory effect of estradiol hormone on the growth of the testicular epithelial tissue.

Table (4) and Figure (8): indicates a notable reduction in the diameter of the seminiferous tubules of the Wister rat testis in the treated group (160.582 ±0.281) (µm) when compared with diameter

(420.96 ±4.89) (µm) in control group, confirming estradiol's inhibitory impact on testicular structure. This finding aligns with Setchell (2016), who noted that histometric alterations in testis frequently indicate impaired spermatogenesis

Our findings provide robust support for the hypothesis that exposure to estrogenic substances during specific developmental stages can alter the hormonal and neuronal landscape, resulting in notable behavioral changes that can impact the reproductive capacity (Ceccarelli et al., 2007). Chaki et al., (2006) who mention the long time estradiol administration in rats due to decreased activity of testicular antioxidant enzymes, such as SOD and catalase, seemingly resulting in heightened oxidative stress Furthermore Oxidative stress and inflammation play a crucial role in the reproductive toxicity linked to endocrine-disrupting chemicals (EDCs) that may impair spermatogenesis and hormonal regulation (**Chen** et al., 2025; Tzouma et al., 2025). These findings support the notion that the testicular pathogenesis of estradiol benzoate treatment could mainly result from changed reproductive hormone levels and increased oxidative stress causing germ cell apoptosis and the eventual loss of germ cells in the seminiferous epithelium (Chaki et al., 2006) .These findings bolster the theory that contact with hormone-disrupting endocrine disruptors is associated with observable and clinically important impacts on human fertility and reproductive health (Tzouma et al., 2025)

Table. (4) Histometric measurement Diameter(µm) of the seminiferous tubules of the testis of the Wister rat at two month of age in control group (non-treated)(G1) and treated group(G2)

Measure / Group	Diameter of the seminiferous tubules (µm)
G1	420.96 ±4.89
G2	160.582 ±0.281
T- test	130.21
P- value	4.49
A highly significant decrease was found in the length value in the treated group G2 compared to the control group G1, indicating a strong effect of the treatment in reducing tissue or longitudinal growth in the sample.	

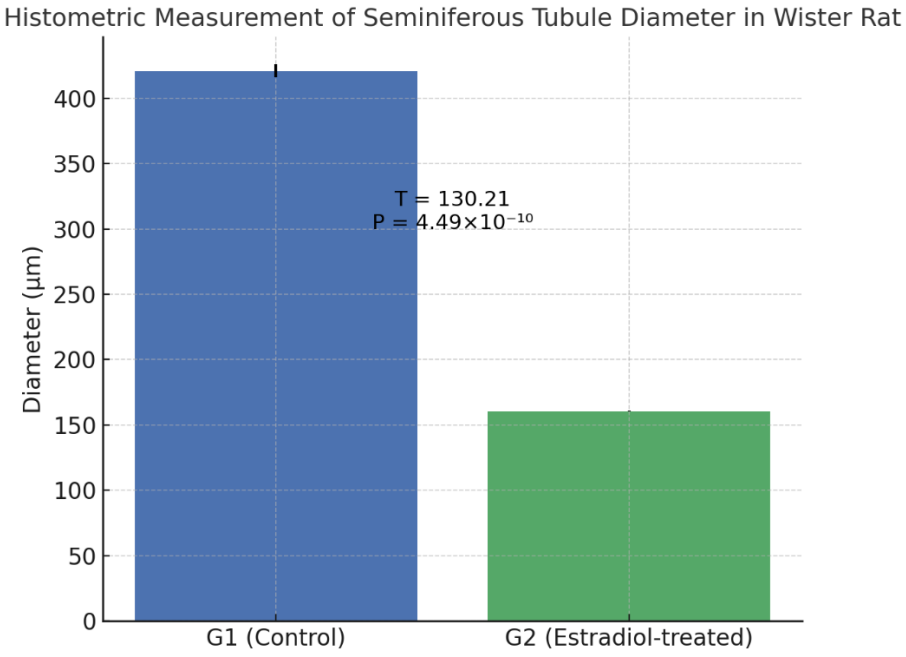

Figure (8) the notable reduction in testis Wister rat seminiferous tubule width in estradiol between the treated group (G2) and the control group (G1).

Conclusion

Findings outlined above have convincingly illustrated that 17ß-estradiol exerts measurable biological effects on male rats, impacting the seminiferous epithelium as a result of elevated testicular oxidative stress and supports the theory that contact with exogenous estrogens negatively impacts male fertility via interconnected mechanisms, such as the reduction of testosterone production, interruption of spermatogenesis, and histopathological and morphological alterations in the testis. The results from the present study will ideally. illuminate the biological impacts that might arise from exposure to endocrine disruptors

Recognition

The College of Veterinary Medicine at the University of Al-Qadisiyah in Iraq is acknowledged by the writers.

Funding

No specific funding was received.

Authors' contributions

All authors have participated equally in the current research.

Conflict of interest

There is no disclosure of the conflict of interest.

Data availability

The data are available via the corresponding author when requested

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