The Pathological Effect of Exogenous Testosterone on the Male Genital Organs of Rat

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ABSTRACT
Testosterone hormone is the primary male hormone which is responsible for regulating sex differentiation, producing male sex characteristics, spermatogenesis and fertility. This study was conducted to investigate the histopathological lesions in the male genital organs (testis and epididymis) of rats associated with subcutaneous administration of therapeutic and toxic dose of testosterone hormone. In this study 30 male rats were divided into three groups. The first and second group was injected with the therapeutic and toxic dose of testosterone hormone respectively, while the control group (third group), was, subcutaneously injected with olive oil only in a daily manner. The period of the experiment was for 12 weeks and at the end of the experiment all animals (Rats) were euthanized and the specimens were collected from the male genital organs (testis and epididymis). The result of histopathological examination showed that administration of exogenous hormone caused moderate loss of spermatogenesis in the therapeutic treated group, while severe damage was seen in toxic treated group as well as degenerative changes of spermatogonia and atrophy of Sertoli and Leydig cells in the both treated groups. Other lesions have been observed in the toxic treated group were nerosis, and atrophy of seminiferous tubules. Moreover, epididymal tubules of this group showed complete loss of spermatocyte and fiber-like projection extended toward the lumen of the tubules compared with the therapeutic dose group that showed moderate hyperplasia of epithelial lining cells of epididymal tubules.

INTRODUCTION
Testosterone is a sex steroid hormone with an important physiological role in male and female, it is implicated in the development of morphological and functional process, which is controlled by different molecular mechanism, (Jaroslava et al., 2011). It is also subjected in the development of primary sexual characteristics, which includes testicular descent, spermatogenesis and increasing libido, (Kalfa et al., 2019). On the other hand testosterone have the ability to regulate secondary male characteristics, include male hair patterns, vocal changes, voice deepening and anabolic effects that include growth spurts in puberty and increases tissue growth at the epiphyseal plate early on and eventual closure of plate later in puberty and skeletal muscle growth, stimulates protein synthesis and erythropoiesis, (Plant and Marshall, 2001). In addition testosterone regulates sex drive and other processes in male and female. The main sit of production of testosterone in men, is localized in the smooth endoplasmatic reticulum of Leydig cells in the testes, (Brown, 1989). In females, testosterone hormone is generated by the ovaries and the cortex of suprarenal glands (Lobotsky et al., 1964; Wu et al., 2010) and some regions of the brain, (Mensah et al., 1996; Matsunaga et al., 2002). In puberty, the hypothalamic pituitary gonadal axis plays an important role in regulating the levels of testosterone hormone and gonadal functions. The hypothalamus secretes GnRH, that effect on the hypothalamo hypophyseal portal system to the anterior lobe of pituitary gland to secretes two hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), these hormones which released through the blood and act on its receptors in the gonads. Luteinizing hormone acts on Leydig cells to increase testosterone production. On the other hand, testosterone control on the secretion through negative feedback, so elevated level of testosterone in the blood lead to cause feedback to the hypothalamus to suppress the secretion of GnRH and also feedback to the pituitary gland to decrease the response to GnRH, (Plant and Marshall, 2001).

MATERIALS AND METHODS
Thirty male rats at age of 21 days weighing approximately 150 gm, were divided into three main groups (n=10 each group) and treated daily for 12 weeks as following: The first group, (therapeutic treated group), received the therapeutic dose of testosterone hormone subcutaneously (0.1 mg) that diluted in olive oil as a vehicle at a dose of 0.1 mg. The second group (toxic treated group) received toxic dose of testosterone hormone subcutaneously diluted in olive oil as vehicles at dose of 0.3 mg. While the third group (control group) received olive oil only. At the end of the experiment (12 weeks) of daily s/c injection, all the experimental animals were euthanized, and the specimens were collected from (testes and epididymis). The specimens were preserved in 10% neutral buffered formalin solution (NBF) and processed according, (Luna, 1968) to prepare for histopathological examination and then stained with Haematoxyllin and Eosine stain (H&E) and then examined by light microscope.

RESULTS
The results of this experiment revealed that exogenous testosterone hormone induced different pathological changes of testicular and epididymal tissues of both group therapeutic and toxic treated group. Most of these changes appeared sever in toxic treated group while moderate in therapeutic treated group. These changes characterized by degenerative changes of spermatocyte in the seminiferous tubules, (Fig 1), and formation of spermatid giant cell (Fig 2). In the same group (therapeutic), other section of testes showed atrophy of...
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Seminiferous tubules with partial loss of spermatozoa (Fig 3). Marked necrosis of seminiferous tubules with irregularity and appearances of multinuclear giant cell with complete atrophy of Leydig cells (Fig 4). Tubular atrophy of seminiferous tubules with reduction of spermatocyte was prominent and atrophy of Sertoli and Leydig cells (Fig 5) and Severe degeneration of spermatogonia and atrophy of Leydig cells (Fig 6). Epididymal tubules also showed partial loss of mature spermatozoa and moderate hyperplasia of epithelial lining cells of epididymal tubules (Fig 7). In the toxic treated group, the lesion appeared more severe than in therapeutic dose, that characterized by necrosis of seminiferous tubules and complete loss of spermatozoa in seminiferous tubules (Fig 8). Marked atrophy of seminiferous tubules and fibrosis of tunica albuginea have been observed in (Fig 9) and the lesion progress to epithelial hyperplasia of epididymal tubules as a finger like shape extend toward the lumen and sloughing and reduction of spermatoocyte (Fig 10). Epididymal tubules appeared empty referred to complete loss of spermatoocyte (Fig 11).

Fig 1: Histopathological section of testis of Rat treated with testosterone hormone (0.1 mg), showed degenerative changes of spermatocyte in the seminiferous tubules (A), (H&E) X100.

Fig 2: Histopathological section of testis of Rat treated with testosterone hormone (0.1 mg), showed vacuolation of spermatozoa (A) and irregular hypereosinophilic elongated spermatid and formation of spermatid giant cells (B), (H&E) X400.
Fig 3: Histopathological section of testis of Rat treated with testosterone hormone (0.1 mg), showed atrophy of some seminiferous tubules (A) with partial loss of spermatozoa (B). (H&E) X100.

Fig 4: Histopathological section of testis of Rat treated with testosterone hormone (0.1 mg), showed, marked necrosis of seminiferous tubules (A), with irregularity and formation of multinuclear giant cells (B), with atrophy of Leydig and Sertoli cells. (H&E) X100.
Fig 5: Histopathological section of testis of Rat treated with testosterone hormone (0.1mg), showed tubular atrophy of seminiferous tubules (A), with reduction of spermatocyte (B) and atrophy of Sertoli cell (H&E) X100.

Fig 6: Histopathological section of testis of Rat treated with testosterone hormone (0.1 mg), showed degeneration of spermatogonia (A), and atrophy of Leydig cells (H&E) X100.
Fig 7: Histopathological section of epididymal tubules of Rat treated with testosterone hormone (0.1 mg), showed partial loss of mature spermatozoa (A) and moderate hyperplasia of epithelial lining of epididymal tubules (B), (H&E) X100.

Fig 8: Histopathological section of testis of Rat treated with testosterone hormone (0.3 mg), showed marked necrosis of epithelial lining cell of seminiferous tubules (A) and complete loss of spermatozoa, (B) (H&E) X100.
Fig 9: Histopathological section of testis of Rat treated with testosterone hormone (0.3mg), showed marked atrophy with irregular edges of seminiferous tubules (A), fibrosis of tunica albuginea (B) (H&E) X100.

Fig 10: Histopathological section of epididymis of Rat treated with testosterone hormone (0.3mg), showed epithelial hyperplasia of epididymal tubules (A) and sloughing (B) and complete loss of spermatocyte (H&E) X100.
DISCUSSION
The results of our experimental study demonstrated that different dose of exogenous testosterone promoted male reproductive toxicity in Rat. In the present study, injection of therapeutic dose of exogenous testosterone hormone has caused degenerative changes of spermatocyte and vaculolation of spermatozoa that lead to suppression of spermatogenesis, that appeared slight in therapeutic treated group and sever in the toxic one. As it's well-known that the hypothalamus secretes (GnRH) , that effect on the anterior lobe of pituitary gland to secrete (follicle stimulating hormone and luteinizing hormone), in the blood stream that effect on the target organs, luteinizing hormone acts on its receptors on Leydig cells of the testes to increase the production of testosterone hormone. Consequently elevated levels of testosterone hormone in the blood due to exogenous hormone, lead to feedback to the hypothalamus to suppress the secretion of GnRH as well as feedback to the pituitary making it less responsive to GnRH stimuli,(Plant and Marshall,2001).In the present experiment the results demonstrated that most of these changes triggered by exogenous testosterone were associated with intra-testicular testosterone this results agreed with (Roth , 2012).who revealed that exogenous testosterone cause suppression of, hypothalamic (GnRH) and pituitary (FSH and LH) production, cause depletion of intra-testicular testosterone and suppression of spermatogenesis. Other scientist (Ericsson et al.,1964;Jia et al.,2001), have supported these findings which referred that the inhibition of the spermatogenesis may be result from the inhibition of LH and FSH hormones responsible for the activity of Sertoli cells ,that lead to suppression of spermatogenesis (azoospermia).Several studies referred that the more severe or chronic reductions in intra-testicular testosterone, lead to generalized reduction in cellularity, as it is clear in this experiment. Therefore the lesion in the toxic treated group demonstrated more severe damage associated with toxic dose,(Dsouza and Parthak,2009).

These changes progress to more degenerative changes of spermatocyte and formation of, (spermatid giant cell), these results also pointed by other author, (Yang et al.,2006), who refer that, the damage of spermatogenesis occurred after three months of treatment, due to detachment of spermatids and spermatocytes, the presence of spermatids, degenerating round spermatids and spermatogenic cells, noticed in the lumen of seminiferous tubules indicate impairment of meiosis and spermatogenesis. These changes observed when the level of gonadotrophin decline, and the process of spermatogenesis is suppressed at both its starting point (conversion from type A to type B spermatogonia) and end point (releasing of elongated spermatids), i.e. spermatiation ,these results improved by (Zhengwei et al ,1998) .Others (Lee and Kinney ,1989).showed that incomplete spermatogenesis and the presence of spermatid giant indicating that there was testicular degeneration occurred in the seminiferous tubules of testis that appeared to be secondary changes resulting from disrupt Sertoli cell. On the other hand seminiferous tubules appeared irregular shape and other were atrophied with atrophic Leydig cells ,these cell responsible to turn cholesterol into testosterone ),these result agree with (Xiao-Wei Zhang et al 2016).The present study demonstrated that the administration of exogenous testosterone have an effect on epididymal tubules cause a pronounced decline in the number of epididymal spermatozoa ,(Roth,2012), due to suppression of testosterone which is necessary for the synthesis and secretion of a number of proteins by the epithelial cells of the epididymis, which are needed for sperm maturation and sperm storage, (Hikim et al.,1985).
Testosterone like other steroid hormone such as estrogen has the ability to induce hyperplasia of the epithelial lining cell of epididymis due to binding to its nuclear receptors, (Milgram et al.,1973) referred that the action of steroid hormones are thought to be mediated through their binding to steroids receptor. In addition testosterone and dihydrotestosterone are ligands of the
nuclear androgen receptors (Askew et al., 2007), so the therapeutic treated group showed slight hyperplasia of the epithelial lining cell due to excessive stimulation of the epithelial lining cell by exogenous testosterone lead to activated receptors which are crucial for the transcription factor that regulates expression of genes included proliferation of cells, metabolism, differentiation and apoptosis, (Nelson et al., 2002). In the toxic treated group the hyperplasia of the epithelia was more prominent in all section of organs of animals treated with toxic dose of testosterone hormone produce (finger like shape) protruded in the lumen, due to excessive dose which activated receptors for the regulation of expression protein, coactivators (Estebanaza et al., 2005). An activated of androgen receptors stimulates an elevated level of intracellular Ca2+ that lead to stimulate multiple protein kinases, that permit protein interactions and triggering crucial signaling cascade, as it is refer by, (Foradori et al., 2010; Baron et al., 2004; Li and Al-Azzawi, 2009).

REFERENCES