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Full Length Research Paper

Histomorphometrical study of seminiferous tubule in rats after used *Tribulus terresteris*

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The aim of the study was to determine the histological and histomorphometrical change of seminiferous tubule in mature and immature wistar rats after using *Tribulus terresteris* (TT). Twenty male wistar rats were selected and randomly divided into four groups: 1) Mature control group (MCG). 2) Mature experimental group (MEG) (orally received 75 mg/kg TT daily for 14 days). 3) Immature control group (ICG). 4) Immature experimental group (IEG) (orally received 75 mg/kg TT daily for 14 days). The number of leydig cells had increment in experimental group when compared with ICG. Result showed that the thickness of the wall of seminiferous tubule in experimental group significantly increased (P < 0.05). Also, TT treatment groups resulted the accumulation of spermatogenic cells were increased in the seminiferous tubule when compared with control group. In addition, sperm was not observed in ICG but sperm were also observed to increase in the treatment groups. It is concluded that TT may improves the sexual activity, increased testestrone by intensification of leydig cells and may caused early puberty in immature rat.

Key words: Histomorphometrical, rat, seminiferous tubule, *Tribulus terresteris*.

INTRODUCTION

The testis contain of many pyramidal compartments called the testicular lobules. Each lobule is occupied by 1 - 4 seminiferous tubules enmeshed in a web of loose connective tissue that is leydig cells. Seminiferous tubul-es produce male reproductive cells, the sperma-tozoa, whereas leydig cells secrete testicular androgens. Seminiferous tubules lined with a complex stratified epithelium that consist of a tunic of fibrous connective tissue, a well-defined basal lamina and a complex germi-nal epithelium (Banks, 1993). TT is a flavnonoid herbal drug that has saponin, essence and steroid.

A lot of various studies were undertaken on increasing testosterone and sexual activity after used TT (Gauthman, 2003, 2008; El-tantawy, 2007; Park, 2006). In addition, the interferon reduced sertoli cells and the thickness of seminiferous tubular epithelium (Natwar, 1995). Administration of various concentration of sodium fluoride showed that it did not have effect on the testosterone and spermatogenesis and it can cause diminishing of capsule of testis with 100 ppm dosage (Sprando, 1997). Also, Sprando et al. (2000) indicated that linen seed decreased the volume of the seminiferous tubules. The aim of the present study was to investigate the effect of TT on histological change and histomorpho-metrical of seminiferous tubule of the testes.

MATERIALS AND METHODS

Ten mature wistar male rats weighing between 200 - 250 g (60 days old) and ten immature wistar male rats weighing between 50 - 75 g (21 days old) were obtained from animal house of Islamic Azad University, Kazerun branch. The animals were divided into four groups:

1) Mature control group.
2) Mature experimental group (orally received 75 mg/kg TT daily for 14 days).
3) Immature control group.
4) Immature experimental group (orally received 75 mg/kg TT daily for 14 days).

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Abbreviation: TT, *Tribulus terresteris.*

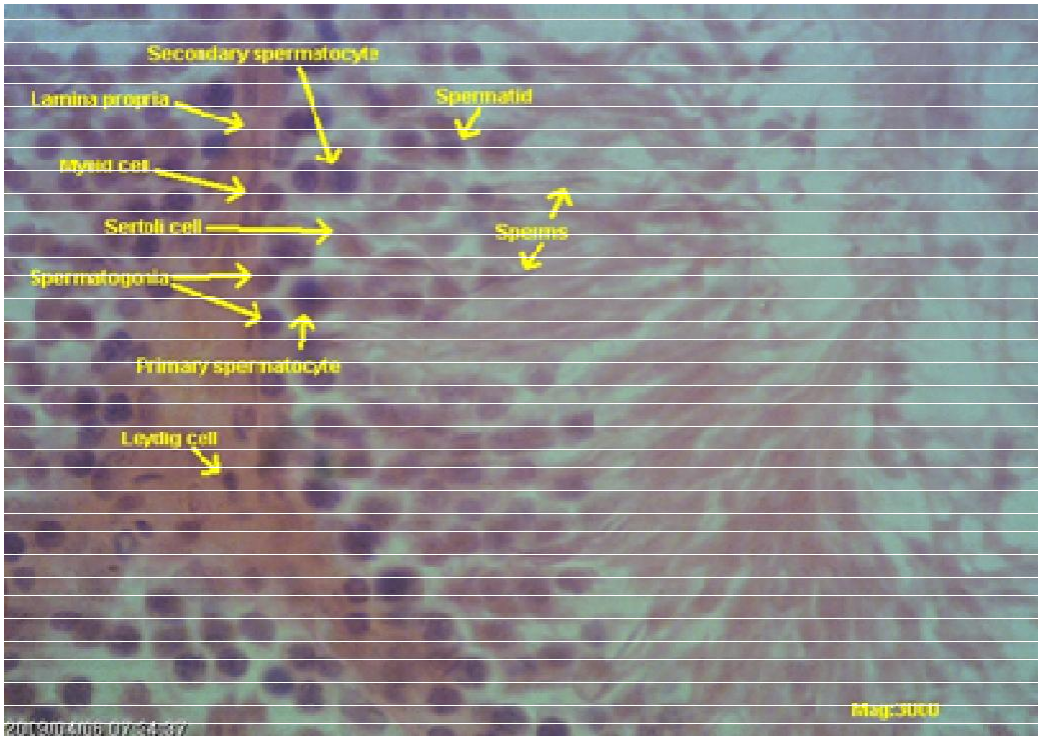

Figure 1. Testicular section from a mature control group shows the spermatogenic cells and histological structure of the seminiferous tubules (H and E ×3000).

All rats fed a standard diet and water. The rats were anesthetized using ether and the peritoneal cavity was opened through a lower transverse abdominal incision. The testes were carefully removed, washed in normal saline solution, blotted and weighed and transferred to 10% formalin buffer for 24 h. Specimens were embedded in paraffin and afterward five-micron thick section were prepared and stained with hematoxylin and eosin (H and E). These sections examined under light microscope and standard micrometry technique. The laboratory care, anesthesia and euthanasia of animals used in this study were performed in accordance with the guide to the care and use of experimental animals.

Statistical analysis

Statistical analysis was performed using SPSS version 16 software. Results were presented as the mean ± SD. Statistical comparisons were made by one way ANOVA. A probability value less than 0.05 was considered statistically significant.

RESULTS

The testes are surrounded by a thick capsule of dense connective tissue, the *Tunica albuginea*. The *Tunica albuginea* is continuous with connective tissue trabeculae. These trabeculae are rather complete septa. The septula testis divides the testicular parenchyma into a varying number of testicular lobules. The intertubular spaces contain loose connective tissue and leydig cells. The leydig cells are large cells with spherical nuclei and acidophilic cytoplasm (Figure 1). The TT is seminiferous tubules lined by the stratified germinal epithelium, surrounded by a lamina propria. Various spermatogenic cells, representing different phases in the development and sertoli cells are located in epithelium of seminiferous tubules (Figure 1). Sperm was not seen in ICG and also, in this group were leydig cells so little (Figure 2). After weighing testes, the mean of weight of testes in the MCG, MEG, ICG and IEG, were 1.56 ± 0.04, 2.14 ± 0.08, 0.8 ± 0.02 and 1.48 ± 0.03 g, respectively, (Table 1). Histomorpho metrical study showed that the mean of thickness of wall of seminiferous tubules in the MCG, MEG, ICG and IEG, were 39.66 ± 4.13, 73.99 ± 17.23, 21.81 ± 2.98 and 55.63 ± 4.39 µm, respectively, (Table 1). In addition, histological study showed that the accumulation of spermatogenic cells and leydig cells in seminiferous tubules were increased in MEG and IEG when compared with MCG and ICG (Figures 2, 3, 4 and 5) . Also, sperm was seen in wall of seminiferous tubules in the IEG after used TT (Figure 4).

DISCUSSION

A herbal flavonoid drug and it contains saponin, steroid and different essence. It has a lot of effects such as increasing testosterone and dihydrotestosterone levels (El-tantawy, 2007; Gauthman, 2003, 2008), preventing cell death and destruction of mitochondrial membrane (Liu, 2008), antimicrobial and antifungal effects (Al_bayati,

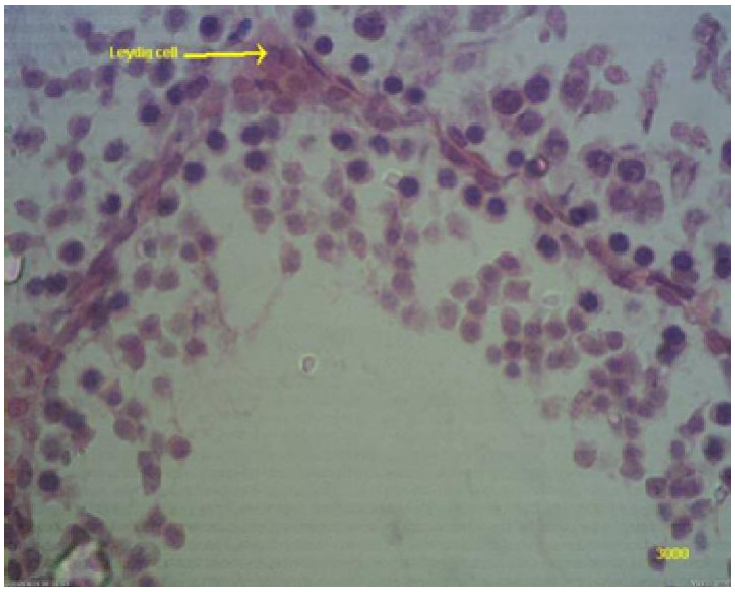

Figure 2. Testicular section from an immature control group shows sperm was not seen and leydig cells were so little (H and E × 3000).

Table 1. Effect of *Tribulus terresteris* on testis weight and thickness of seminiferous tubules (Mean ± SD).

Group	Weight of testis (gram)*	Seminiferous tubules thickness(µm)*	
Mature	Control (MCG)	1.56 ± 0.04 a,c	39.66 ± 4.13 a,c
	Experimental (MEG)	2.14 ± 0.08 a,d	73.99 ± 17.23 a,d
	Control (ICG)	0.8 ± 0.02 b,c,d	21.81 ± 2.98 b,c,d
Immature	Experimental (IEG)	1.48 ± 0.03 b,d	55.63 ± 4.39 b,

MCG, Mature control group; MEG, mature experimental group; ICG, immature control group; IEG, immature experimental group. experimental groups received orally 75 mg/kg TT daily for 14 days.
*: The same superscript letter (a, b, c, d) in each column shows significant difference between groups ($P < 0.05$).

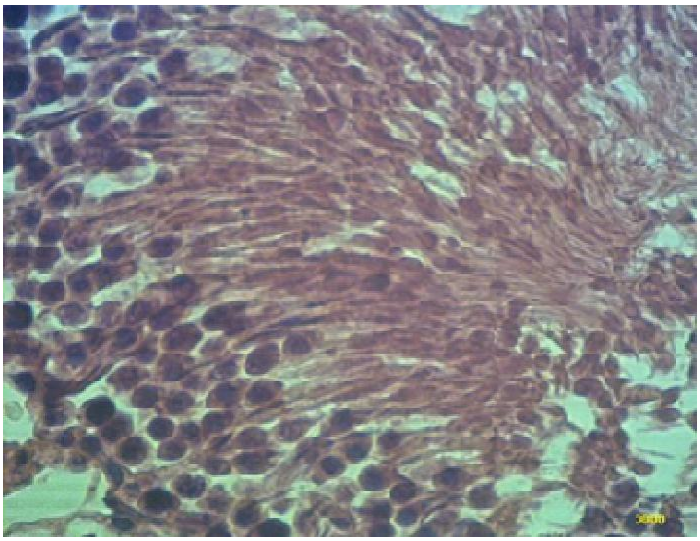

Figure 3. Testicular section from a mature experimental group shows increase the number of spermatogenic cells and leydig cells of seminiferous tubule (H and E × 3000).

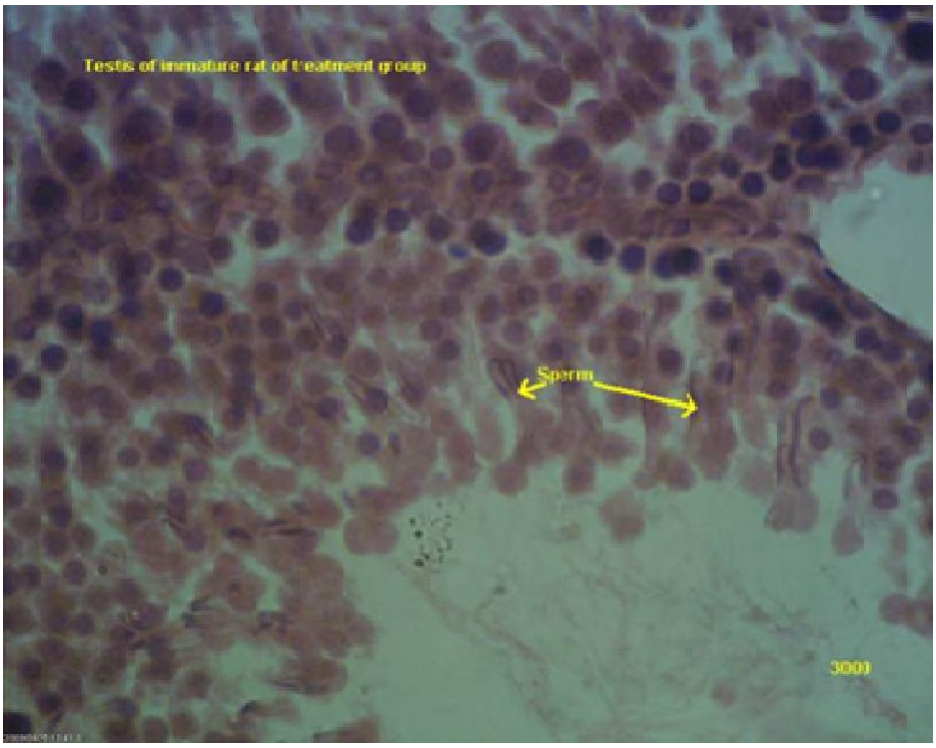

Figure 4. Testicular section from an immature experimental group shows increase the number of spermatogenic cells and leydig cells in seminiferous tubule, Sperms were seen (arrows) (H and E × 3000).

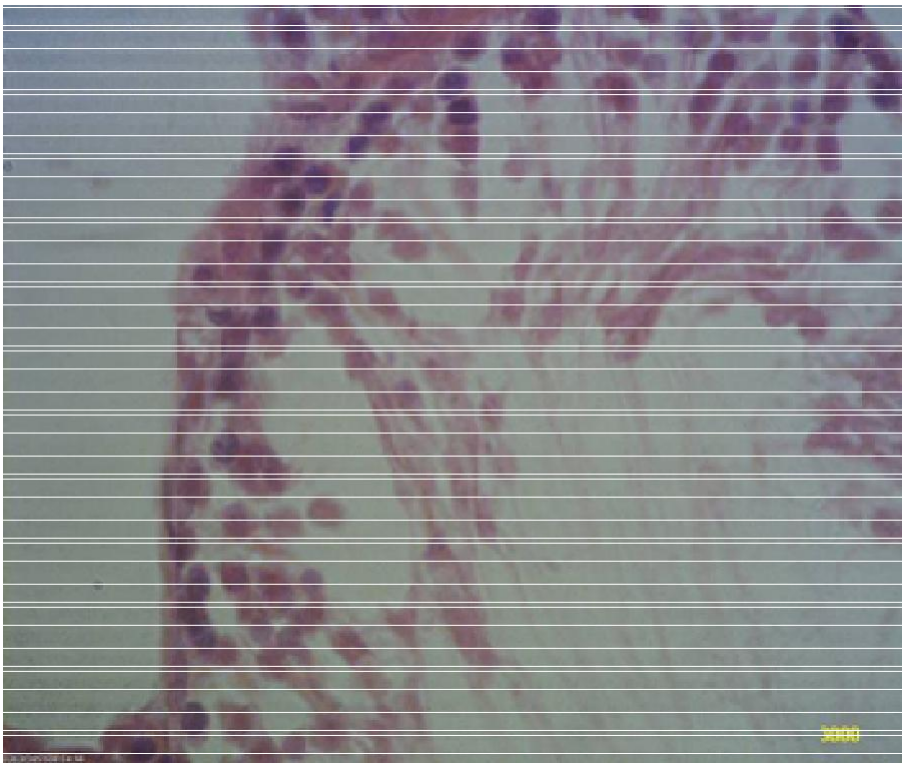

Figure 5. Testicular section from a mature control group shows the accumulation of spermatogenic cells of seminiferous tubule (H and E × 3000).

2008), decreasing blood sugar in diabetic patients, cholesterol and triglyceride (Chu, 2003; Li, 2002; El-tantawy, 2007), increasing muscular and fat mass (Rogerson, 2007), increasing androgen and sexual desires (El-tantawy, 2007), repairing of left ventricle of heart (Guo, 2007), increasing blood pressure of penile artery (Park, 2006) and increasing melanocyte-stimulating hormone (Yang, 2006). The results of our study showed that the thickness of wall of seminiferous tubules were significantly increased in experimental groups as compare to control groups ($P < 0.05$) but, there was no significant difference between MCG and IEG ($P > 0.05$). In addition, sperm was seen and leydig cells were increased after using TT in immature rats.

It seems that TT cause early puberty and it can increase testosterone levels and sexual desires. Our findings are essentially in agreement with El-tantawy et al. (2007) and Gauthman et al. (2003, 2008) findings. Significant increase was observed in weight of testes in experimental groups as compared with control groups ($P < 0.05$), but no significant difference between MCG and IEG was observed ($P > 0.05$). Maybe, these result due to increasing in function and compactness of cells of seminiferous tubules and consequently we have an increased excretion in these tubules. We concluded that TT may cause early puberty and it may increase testosterone levels and it can increase compactness of spermatogenic cells and sperms.

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