

The effect of the *Tribulus terrestris* extract on spermatogenesis in the rat

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Abstract

Introduction:

According to our antecedents, in addition to other pharmacological properties, *Tribulus terrestris* increases sexual functioning and libido in men. Due to the fact that the benefits of herbal drugs should be approved clinically in the laboratory, the present study aimed to investigate the effect of *Tribulus terrestris* extract on the primary spermatocyte in rat.

Material and Methods:

In this experimental study, thirty five mature male Wistar rats with average weight of 180 ± 10 g and age of 2-3 months were randomly divided into five groups of seven each. Group I served as a control and group II as a experiment group (normal saline, 2.5 ml) and groups III, IV and V were treated with three different doses of oral TT extract (2.5, 5 and 10 mg/kg body weight, respectively) once daily for 8 weeks. After the last treatment, the rats were sacrificed and their testis was removed, fixed and studied using light microscope. The data were analyzed using Anova.

Results:

The results of this study showed that the mean number of primary spermatocytes in the 3rd experimental group (10 mg/kg/body) increased significantly compared with the control group ($P < 0.01$). But *Tribulus terrestris* extract had no effect on the mean number of primary spermatocytes in the other experimental groups.

Conclusion:

The above results showed that *Tribulus terrestris* can probably balance the functions of the male reproductive system and can be used in treatment of male infertility, while effecting the testis spermatocyte.

Keywords: Spermatocyte, Saponin, Fertility, Wistar Rats

Introduction:

Tribulus terrestris known as Puncture vine is a native yearling lying plant which has been widely spread in Mediterranean area and warm areas of Europe, Asia, America, Africa and Australia (1). It has been widely used in the traditional medicines of China, India, Iraq, Bulgaria, South Africa and also Iran. Studies show that *Tribulus terrestris*

contains steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, nitrate potassium, aspartic acid and glutamic acid (2). This plant has several advantages including antimicrobial, antibacterial, antioxidant and antitoxic activities used in the treatment of cardiovascular diseases, diabetes, tumors, articular pains and respiratory diseases (3-7).

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Tribulus terrestris increases body ability to produce muscular mass and physical strength. Furthermore, it causes production of red blood cells and improvement in circulation and oxygen transportation (8). Long-term use of *Tribulus terrestris* results in dilatation and improvement of coronary arteries without any side effects (9). *Tribestan* is a component of *Tribulus terrestris* which increases libido in addition to preventing reluctance, infertility and menopausal disorders (10). Researchers have shown that dioscin present in *Tribulus terrestris* increases male sexual ability by increasing free testosterone levels and balancing levels of estrogen, progesterone and pregnenolone (11). Since this plant contains protodioscin and saponins which increase levels of testosterone and luteotropic hormone (LH), it has been used for the treatment of sexual impotence in the traditional medicines of China and India from long ago (12). In a study, it has been reported that *Tribulus terrestris* has no effect on organs sensitive to endocrine glands such as prostate, seminal vesicle, uterus and vagina of male and female rats (13). Besides, using *Tribulus terrestris* in addition to other herbal drugs has improved erection and sexual behavior in rat (14). In another study, *Tribulus terrestris* has increased sexual hormones in rat, rabbit and primate (15). According to the findings of these studies showing *Tribulus terrestris* to increase sexual hormones, to improve sexual behavior and erection, it seems that this plant can be effective in spermatogenesis as well. Since there is no report about the effect of *Tribulus terrestris* extract on testis, the present study aimed to evaluate the effect of *Tribulus terrestris* on testis tissue of rats.

Material and methods:

In an experimental study, 35 mature male Wistar rats aged 2-3 months with a mean weight of 180 ± 10 grams were used. The rats

were provided from Razi institute. This study was conducted in the Animal House of Shiraz University of Medical Sciences in 2011. During the study, a lighting condition of 12 hour light and 12 hour dark, at a room temperature of 22-24 ° C and humidity of 55-60% was set. All of the studied animals were sacrificed in accordance with the Animal Protection Act (16).

The rats were randomly divided into five groups of seven; Group I served as the control and group II as the experiment group (normal saline, 2.5 ml) and groups III, IV and V were treated with three different doses of oral *Tribulus terrestris* extract (2.5, 5 and 10 mg/kg body weight, respectively) once daily for 8 weeks. One day after receiving the last dose of the extract, the rats were sedated by ether and their testes were removed by opening the peritoneal area through an abdominal transverse incision. Then, the testes were placed in formalin 10%. After tissue fixation and molding, tissue cut with thickness of 5 micron were produced and colored with hematoxylin-eosin. The produced tissue cuts were studied under light microscope. The primary spermatocytes were calculated in transverse sections of seminiferous with the same number of sections (10 sections in each group) in all of the groups.

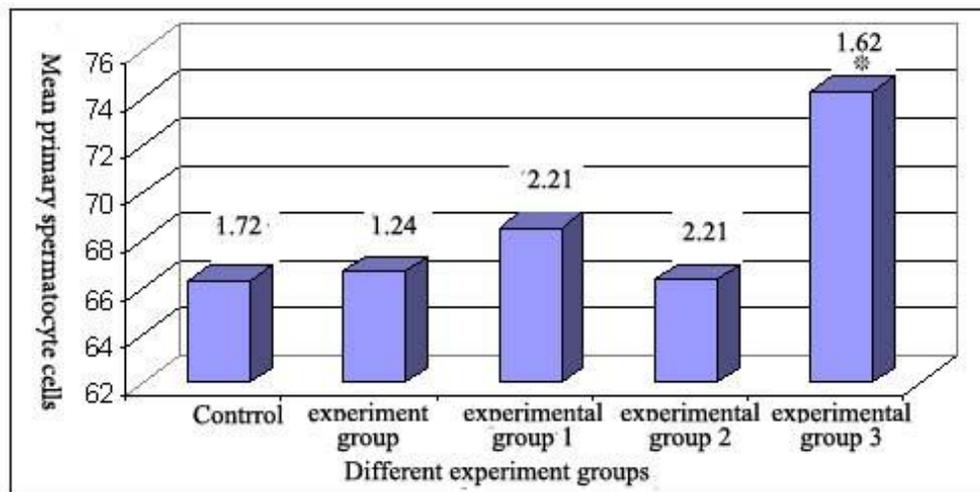
Method of producing *Tribulus terrestris* extract: *Tribulus Terrestris* plant was purchased from grocery approved by Shiraz University of Medical Sciences. The fruit of the plant was powdered by a machine. An amount of 100 grams of the powder was kept in 80cc of hydroalcohol 70% in percolation system for 72 hours in the laboratory temperature and then the extract was taken. By separating the extract solvent using bain de Marie and drying it in vacuum in a desiccator machine for 24 hours, 10.5 grams of crystallized dried extract was obtained.

Data analysis: the collected data in each of the groups were analyzed by one-way ANOVA using SPSS software with $P < 0.01$ as the level of significance.

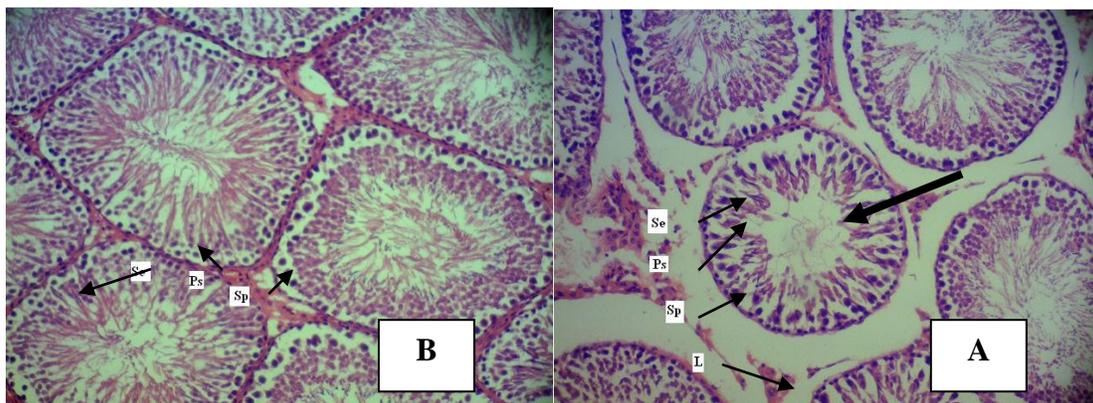
Results:

The result of evaluating the 35 rats has been shown in figure 1. The mean number of the primary spermatocytes in the 3rd

experimental group (with the dose of 10 milligram per kilogram) has significantly increased compared with the control group ($P < 0.01$). However, *Tribulus terrestris* extract did not significantly increase the mean number of the primary spermatocytes in the 1st and the 2nd experimental groups compared with the control group.



Graph 1: the effect of different doses of *Tribulus terrestris* extract on the mean number of the primary spermatocytes in the rats. The mark * shows significant difference ($P < 0.01$) and the number on the top of each column indicates its standard error.



A) The control group in the magnification of $\times 400$

B) The 3rd experimental group (10mg/kg) in the magnification of $\times 400$

Figure 1: photomicrograph of the primary spermatocytes in the transverse section of seminiferous tubules. Sp: spermatogonia, Ps: primary spermatocyte, Se: sertoli, L: leydig. Look at the empty spermatids in the tubule in figure A.

Discussion:

In the present study, the mean number of the primary spermatocytes in the 3rd experimental group (dose of 10 mg/kg) significantly increased compared with the control group ($P < 0.01$). Today, extensive researches have been performed about the use of medicinal plant in the treatment of impotence. According to the books of traditional medicine, use of medicinal plants might improve fertility (17). Studies have shown that alcoholic extract of some parts of a plant in the family of *Tribulus terrestris* with the dose of 50 mg/kg could significantly increase serum level of free testosterone in body. Furthermore, this extract has an Aphrodite activity which probably increases androgens (18). Another study on sheep showed that using 1.5 grams of *Tribulus terrestris* extract for 40 days increased spermatogenesis in sheep during breeding season. Moreover, the number of spermatozoides, sperms viability duration and motility increased and in the newborn sheep from those treated with *Tribulus terrestris* extract a significant improvement in libido and sexual behavior was observed (19). In addition, *Tribulus terrestris* extract has a positive effect on the qualitative and quantitative features of sperms, level of total cholesterol and increase in ejaculation volume and spermatozoides motility in birds as it can decrease 92.4% of serum total cholesterol compared with the control group (20). Studies show that *Tribulus terrestris* extract could improve erection and sexual behavior in rat and increase sexual hormones in rat, rabbit and primate (14, 15). The results of this study showed that the mean number of primary spermatocyte in the experimental group 3 (dose of 10 mg/kg) significantly increased compared to the control group ($P < 0.01$). Follicular-stimulating hormone (FSH) has a direct effect on spermatocyte production so that with decreasing and increasing of the

secretion of this hormone, the number of these cells also decrease and increase. In a study to evaluate the role of FSH and testosterone on spermatogenesis and sertoli cells apoptosis, it was found that this hormone itself has an intensifier role in the process of spermatogenesis. Additionally, testosterone causes delayed sertoli cell death which causes changes in the whole process of spermatogenesis [21]. Thus, the increase in primary spermatocyte in the early and middle stages of spermatogenesis is due to significant increase in Testosterone level and is highly independent of ovulation-stimulating hormone. On the other hand, estradiol glycosides in *Tribulus Terrestris*, among which protodioscin is the most important one, increase the testosterone level. The natural estradiol in this compound may act as a mediator to facilitate the androgen production from estradiol [22] and thus may increase testosterone levels. This hormone itself also results in increased spermatogenesis

The studies show that *Tribulus Terrestris* plant increases secretion of luteotropic hormone from pituitary gland due to containing saponins. luteotropic hormone is also a special stimulant for production of testosterone and hence can improve sexual function in forms of increased sperm production, improved erectile function and increased libido [23]. Furostanol is one of the saponins in *Tribulus Terrestris* with stimulant effect on spermatogenesis. This material significantly improves the quality and quantity of sperm [24].

Conclusion: Generally, according to the results of this study, only dose of 10 mg/kg will increase the mean number of primary spermatocytes in rats. Therefore, it is recommended to investigate the effects of this plant in higher doses and on sterilized rats.

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