Global Veterinaria 10 (1): 13-17, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.10.1.7158

Study on the Effects of Various Doses of Tribulus Terrestris Extract on Epididymal Sperm Morphology and Count in Rat

[1]Ramin Kaffashi Elahi, [2]Sirousrafiy Asl and [2]Farhad Shahian

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine,
Tabriz Branch, Islamic Azad University, Tabriz-Iran
[2]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz-Iran

Abstract: Tribulus Terrestris (TT) is a plant of tropical regions, traditionally used as aphrodisiac, analgesic and diuretic agent. Studies showed that TT extract produces significant aphrodisiac effects and increases testosterone levels. Nowadays TT is a major part of most aphrodisiac drugsand its cardiac effects in long time use are unclear. The purpose was assessment of effects of various doses of Tribulus Terrestris extract on epididymal sperm morphology and its quantity in rat. 21 male wistar rats aged 3-4 month-old weighting 250-350 g were selected by chance. Oral solution of Tribulus Terrestris was prepared and after approving by pharmaceutical center of Tabriz University and after the 2 weeks adaptation was administrated at the dose of 5mg/kg daily. Rats were divided into the 3 identical groups of 7 rats in each. Treatment 1 received normal dose of drug (5 mg/kg) and treatment 2 received the double dose of drug. At the end, rats were constrained and anesthetized by xylazine and ketamine intraperitoneally at the dose of 5 and 60 mg/kg respectively. One of the testis and epididymis were removed at once. The contents of epididymis were extracted by solution Ham's F-10 and transferred on the plates which were in the incubator. Then, plates were stained by Eosin and Nigrosin method and assayed microscopically. Results showed the effectiveness of Tribulus Terrestris extract on sexual dysfunction in experimental rats. On the basis of our results, we concluded that Tribulus Terrestris extract has the potential to be used as a safe therapeutic alternative to current modalities for the management of sexual dysfunction in males.

Key words: Tribulus Terrestris · Sperm · Morphology · Infertility · Rats

INTRODUCTION

The reproductive system or genital system is a system of organs within an organism which work together for the purpose of reproduction. The major organs of the human reproductive system include the external genitalia (penis and vulva) as well as a number of internal organs including the gamete producing gonads (testicles and ovaries)[1].

Many factors are involved in the process of conception and the ensuing pregnancy, including psychological, anatomical and immunological factors, which affect both men and women. According to previous work [2,3], 40-50% of infertility cases are the results of male infertility. This is most commonly caused by varicoceles, infections of the testes, immunological disorders and sperm abnormalities. Proper spermatogenesis, including good sperm morphology and motility, as well as normal sexual functions or libido is important for successful conception [4].

Tribulus Terrestris L. extract, a non-hormonal plant-derived extract, has been successfully used in Europe and Asia to treat sexual dysfunctions. Active components of this extract were determined to be a furostanolsaponin, named protodioscin [5]. Oral administration of this extract to laboratory animals resulted in the stimulation of spermatogenesis and the proliferation of the spermatogonia, which involved cell divisions of the spermatocytes and spermatids.

Corresponding Author: Ramin Kaffashi Elahi, Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz-Iran.

These increases in cellular divisions were not accompanied by the increase of the diameter of the seminiferous tubules[6]. In addition to the increased mitotic activity of the spermatozoa and the increased number of Sertoli cells, sperm viability and survival were also significantly increased. Detailed clinical trials found that protodioscin was not toxic and had no undesirable side-effects. The objective of present study was to assessment of effects of various doses of Tribulus Terrestris extract on epididymal sperm morphology and its quantity in rat.

MATERIALS AND METHODS

This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center.21 male wistar rats aged 3-4 month-old weighting 250-350 g were selected randomly. All animals were kept at room with 22-27 °C temperature and at a natural photoperiod (12/12 darkness/lightness) for 2 week before experiment execution for adaptation. A commercial balanced diet and tap water were provided ad libitum (Fig 1 and 2).

In continue, oral solution of Tribulus Terrestris was prepared and after approving by pharmaceutical center of Tabriz University and after the 2 weeks adaptation was administrated at the dose of 5mg/kg daily. Due to its bitter taste, were mixed with some sugar. The administration route was orally and through gavage. Control group received no treatment with exception of distilled water and sugar.

Rats were divided into 3 identical groups of 7 rats in each. Treatment 1 received normal dose of drug (5 mg/kg) and treatment 2 received the double dose of drug. The duration of experiment was 8 weeks. After 8 weeks, rats were moved to the operation saloon of veterinary faculty. The equipments needed for staining and assessment of sperms were kept in the incubator at 37 °C for preventing of heat stress and bias. At the end, rats were constrained and anesthetized by xylazine and ketamine intraperitoneally at the dose of 5 and 60 mg/kg respectively. One of the testis and epididymis were removed at once. The contents of epididymis were extracted by solution Ham's F-10 and transferred on the plates which were in the incubator. Then, plates were stained by Eosin and Nigrosin method and assayed microscopically.

Fig. 1: Cages and place in which rats were kept.

Fig. 2: Food and water were provided *ad libitum*.

RESULTS

Live Sperms Count: Figure 3 shows that,the quantity of live sperms in treatment group 2 and normal controls is less than treatment group 1 (Fig. 3 and 4).

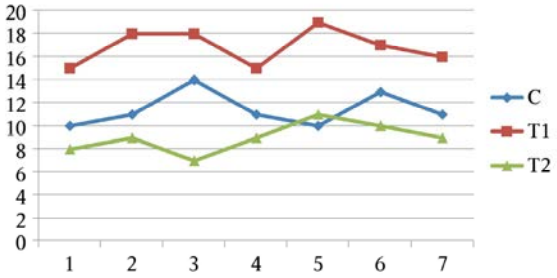

Fig. 3: Average number of live sperms in 4 randomized microscopic field.

Fig. 4: microscopic view of live sperms.

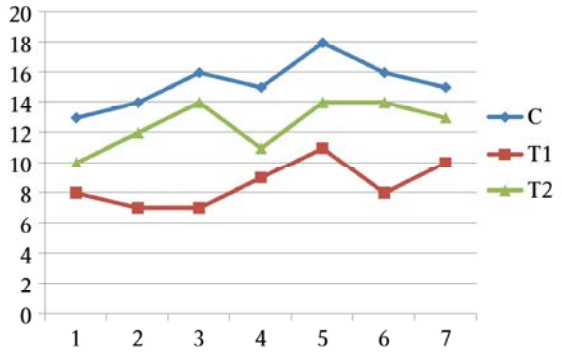

Fig. 5: Average number of sperms with proximal tail droplets in 4 randomized microscopic field.

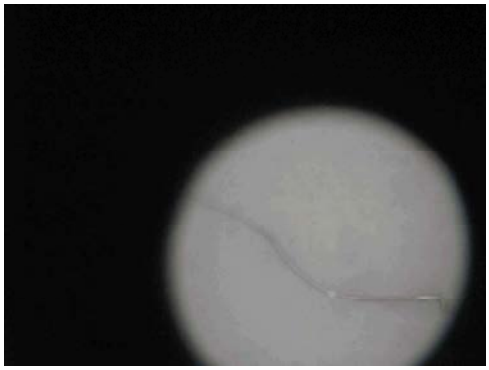

Fig. 6: Microscopic view of sperms with proximal tail droplets

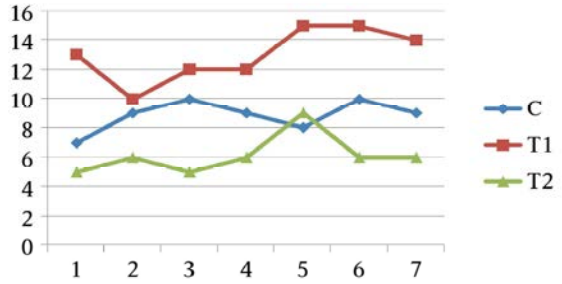

Fig. 7: Average number of sperms with distal tail droplets in 4 randomized microscopic field.

Proximal Tail Droplets: Figure 5 shows that sperms maturity in treatment group 2 and normal controls is less than treatment 1 (Fig. 6).

Distal Tail Droplets: Figure 7 shows that sperms with distal tail droplets in treatment group 2 and normal controls is less than treatment 1 (Fig. 8).

Malformed Sperms Count: Figure 9 shows that malformed sperms count in treatment group 2 and normal controls is more than treatment 1 (Fig. 10).

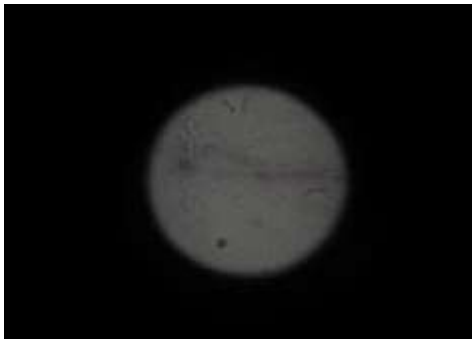

Fig. 8: Microscopic view of sperms with distaltail droplets

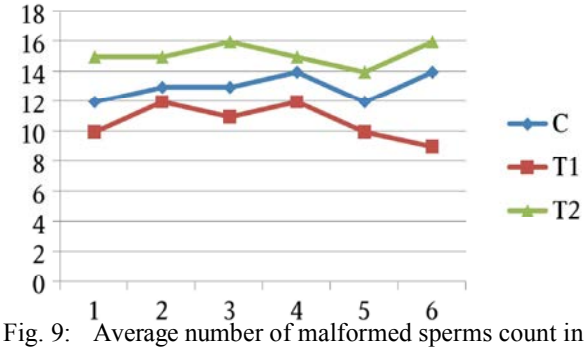

Fig. 9: Average number of malformed sperms count in 4 randomized microscopic field.

Fig. 10: Microscopic view of sperms with Malformations.

DISCUSSION AND CONCLUSION

The sperm cell count, motility, live/dead sperm cell ratio, morphology and the seminal volume were used in this study to evaluate the effect of prolonged administration of Tribulus Terrestris on male reproductive system using the Wistar rat as animal model. These andrological parameters are usually evaluated to determine the fertility of a male subject [7]. When critical percentages (i.e. 10%) of sperm cell abnormalities are present in the semen, the male subject is usuallyconsideredinfertile [8].

Alteration of sperm cell morphology caused by Tribulus Terrestris in this study can be grouped into primary or secondary abnormalities according to the classification byNoarkes*et al*.[9]. It was found that that Tribulus Terrestris was not able to stimulate endocrine sensitive tissues such as the prostate, seminal vesicle, uterus and vagina in Wistar rats, indicating lack of androgenic and estrogenic activity *in vivo*. We also showed a positive effect of TT administration on rat sperm production, associated with unchanged levels of circulating androgens [10].

Findings of Singh *et al.* [6] validate the traditional use of T. terrestris as a sexual enhancer in the management of sexual dysfunction in males.

The review of Clément *et al*. [11] concentrates on the use of distinct plants as feed supplements rather than on ways to treat deficiencies and imbalances in energy or macro-and micronutrients. They found that feeding of maca (*Lepidium meyenii*) and khat (*Catha edulis*) has been shown to positively affect sperm production and quality in animals. Some evidence points to favourable effects of leucaena (*Leucaena leucocephala* and *Leucaena pallida*), sesbania (*Sesbania sesban*), pomegranate (*Punica granatum*), tomato (*Solanum lycopersicum*) and amaranth (*Amaranthushypoc hondriacus*) as well, but studies are either superficial or results are partially contradictory. Finally, the review considers the potential usefulness of medicinal herbs [11].

In another study [12] the feeding mixture for the experimental group was enriched with PHE, which was prepared from Eurycomalongifolia, Tribulus Terrestris and Leuzeacarthamoides. In their study samples of ejaculate were collected weekly. Libido was evaluated according to a scale of 0-5 points. Semen volume, sperm motility, percentage of viable spermatozoa, sperm concentration, morphologically abnormal spermatozoa, daily sperm production and sperm survival also were assessed. They analysed amounts of mineral components and free amino acids in seminal plasma. Significant differences they were found in these parameters: libido (4.05 ± 0.22 vs 3.48 ± 0.78; $p < 0.001$), semen volume (331.75 ± 61.91 vs 263.13 ± 87.17 g; $p < 0.001$), sperm concentration (386.25 ± 107.95 vs 487.25 ± 165.50 × 10(3) / ml(3); $p < 0.01$), morphologically abnormal spermatozoa (15.94 ± 11.08 vs 20.88 ± 9.19%; $p < 0.001$) and Mg concentration (28.36 ± 11.59 vs 20.27 ± 13.93 mg/mlp< 0.05). The experimental group's libido was increased by 20% in comparison with the beginning of the experiment. Results of their study showed positive effect of PHE on libido and some parameters of boar semen quality which is compatible with our research's results.

T. terrestris has been shown to enhance sexual behavior in an animal model.It appears to do so by stimulating androgen receptors in the brain [13]. T. terrestris is now being promoted as a booster for the purpose of increasing sex drive. Its use for this purpose originated from a Bulgarian study conducted in the 1970s, which found effects on free testosterone and luteinizing hormone in men belonging to infertile couples. A research review conducted in 2000 stated that the lack of data outside of this study prevents generalizing to healthy individuals [14].

Animal studies in rats, rabbits and primates have demonstrated that administration of Tribulus Terrestris extract can produce statistically significant increases in levels of testosterone, dihydrotestosterone and dehydroepiandrosterone and produces effects suggestive of aphrodisiac activity. On the other hand, one recent study found that T. terrestris caused no increase in testosterone or LH in young men and another found that a commercial supplement containing androstenedione and herbal extracts, including *T. terrestris*, was no more effective at raising testosterone levels than androstenedione alone [5, 13, 15, 16].

The active chemical in *T. terrestris* is likely to be protodioscin (PTN). In a study on mice, T. terrestris was shown to enhance mounting activity and erection better than testosterone cypionate; however, testosterone cypionate is a synthetic ester of testosterone engineered for its longer activity, rather than an immediate effect. Testosterone cypionate has a half-life of 8 days and is administered every 2-4 weeks in humans for testosterone replacement. The proerectile aphrodisiac properties were concluded to likely be due to the release of nitricoxide from the nerve endings innervating the corpus cavernosum penis. Also, T. terrestris was shown to have strong inhibitory activity on COX-2 [17-19].

The present study demonstrates the effectiveness of Tribulus Terrestris extract on sexual dysfunction in experimental rats. On the basis of our results, we conclude that Tribulus Terrestris extract has the potential to be used as a safe therapeutic alternative to current modalities for the management of sexual dysfunction in males.

REFERENCES

1. Thaddeus, M., 1972. Advances in male reproductive physiology. Fertility and Sterility, 23: 699-707.
2. Jaffe, S.B. and R. Jewelewicz, 1991. The basic infertility investigation. Fertility and Sterility, 56: 599-613.
3. Bayer, S.R., 1993. *In vitro* fertilization and male factor infertility: a critical review and update. Assisted Reproduction Review, 3: 244-251.
4. Liu, D.Y. and H.W.G. Baker, 1988. The proportion of human sperm with poor morphology but normal intact acrosomes detected with pisumsativum agglutinin correlates with fertilization *in vitro*. Fertility and Sterility, 50: 288-293.
5. Neychev, V.K. and V.I. Mitev, 2005. The aphrodisiac herb Tribulus Terrestris does not influence the androgen production in young men. Journal of Ethnopharmacology, 101(1-3): 319-23.
6. Singh, S., V. Nair and Y.K. Gupta, 2012. Evaluation of the aphrodisiac activity of Tribulus Terrestris Linn.in sexually sluggish male albino rats. J PharmacolPharmacother, 3(1): 43-7.
7. Garner, D.L. and E.S.E. Hafez, 1993. Spermatozoa and seminal plasma. In E.S.E. Hafez (eds.) Reproduction in Farm animals (6th ed.) Lea and Febiger, Philadelphia, USA., pp: 165-187.
8. Zemjanis, R., 1977. Collection and evaluation of semen. In: Diagnostic. and Therapeutic Techniques in Animal Reproduction. william and Wilkins company, Baltimore, USA, pp: 242.
9. Noarkes, D.E., T.J. Parkinson, G.C.W. England and G.H. Arthur, 2004. Normal reproduction in male animals. In: Arthur's Veterinary Reproduction and Obstetrics. 8th edn. Saunders Publishers, Edinburgh, pp: 673-694.
10. Martino-Andrade, A.J., R.N. Morais, K.M. Spercoski, S.C. Rossi, M.F. Vechi, M. Golin, N.F. Lombardi, C.S. Greca and P.R. Dalsenter, 2010. Effects of Tribulus Terrestris on endocrine sensitive organs in male and female Wistar rats. J. Ethnopharmacol. Jan., 8; 127(1): 165-70.
11. Clément, C., U. Witschi and M. Kreuzer, 2012. The potential influence of plant-based feed supplements on sperm quantity and quality in livestock: a review. Anim Reprod Sci., 132(1-2): 1-10.
12. Frydrychová, S., L. Opletal, K. Macáková, A. Lustyková, M. Rozkot and J. Lipenskı, 2011. Effects of herbal preparation on libido and semen quality in boars. ReprodDomest Anim., 46(4): 573-8.
13. Gauthaman, K., P.G. Adaikan and R.N. Prasad, 2002. Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats. Life Sciences, 71(12): 1385-96.
14. Bucci, L.R., 2000. Selected herbals and human exercise performance. The American Journal of Clinical Nutrition, 72(2 Suppl): 624S-36S.
15. Gauthaman, K. and A.P. Ganesan, 2008. The hormonal effects of Tribulus Terrestris and its role in the management of male erectile dysfunction-an evaluation using primates, rabbit and rat. Phytomedicine, 15(1-2): 44-54.
16. Brown, G.A., M.D. Vukovich and T.A. Reifenrath, 2000. Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men. International Journal of Sport Nutrition and Exercise Metabolism, 10(3): 340-59.
17. Gauthaman, K., A.P. Ganesan and R.N. Prasad, 2003. Sexual effects of puncturevine (Tribulus Terrestris) extract (protodioscin): an evaluation using a rat model. Journal of Alternative and Complementary Medicine, 9(2): 257-65.
18. National Institutes of Health, http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=2268. Retrieved April 24, 2010.
19. Hong, C.H., S.K. Hur, O.J. Oh, S.S. Kim, K.A. Nam and S.K. Lee, 2002. Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. J. Ethnopharmacol., 83(1-2): 153-159.