ORIGINAL ARTICLE

Effects of Puncturevine (TT) on Leydig Cells of Prepubertal Albino Rats

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ABSTRACT

Puncturevine (Tribulus terrestris) is suggested to increase the concentration of testosterone and luteinizing hormone in rats. These hormones are crucial for the development of male germ cells and also for the growth of the somatic cell types required for normal testicular function; these cells include interstitial cells of Leydig. We, therefore, planned this study to examine its effect on Leydig cells using prepubertal rats as an experimental animal. 20 male rats of 14 days of age were divided randomly were into two groups of 10 pups each (A: control and B: experimental). Experimental group received Tribulus terrestris extract in a single oral dose of 70 mg/kg, daily for 20 days starting at 2 weeks of age, whereas, control group received equal amount of weight related distilled water for the same duration. Each animal was sacrificed at the end of experimental period. Testes were moved out of the body cavity, fixed in Bouin's fixative, embedded in paraffin and 4µm thick sections were obtained. The sections were stained with H & E and PAS and examined with a light microscope at different magnifications. Spherical and ovoid Leydig cells per interstitial space of testis were counted. Statistical analysis was done using independent-samples t test. Rats receiving Tribulus terrestris extract showed statistically significant increase in mean number of Leydig cells per interstitiaI space of testes of animals in the experimental group than that in the control ($p<0.014$).

Keywords: Tribulus terrestris, Leydig cells, testes

INTRODUCTION

Spermatogenesis in mammals is dependent upon a group of hormonal messengers which include gonadotrophins, follicle stimulating (FSH) and luteinizing hormones (LH), and the androgens. Each of these hormones takes part in the normal functioning of the seminiferous epithelium. These hormones are crucial for the development of male germ cells and also for the growth of the somatic cell types required for normal testicular function. These cells include interstitial cells of Leydig, which lie in the interstices between the seminiferous tubules and whose primary function is to secrete testosterone. Leydig and Sertoli cells are direct targets for LH and FSH that exert stimulatory effect on spermatogenesis; these hormones are glycoprotein in nature, and are secreted by the anterior pituitary; they act directly on the testes to stimulate production and release of testosterone by Leydig cells and androgen receptor protein by Sertoli cells[1]. Traditional herbs have emerged in the past few years as an 'instant' treatment for sexual and erectile dysfunctions[2]. Tribulus terrestris is one such herbal remedy which in conventional Chinese pharmaceuticals, is used for treating pruritis, edema and tracheitis. In Iranian folk medicine it has been used for relieving fever and control of rheumatic pain.[3] Tribulus terrestris extract has also been used traditionally to treat ocular inflammation.[4] The seeds of this plant are used to treat hemorrhages, kidney stones and gout. Saponins from Tribulus terrestris have hypoglycemic effect.[5]. Tribulus terrestris has large number of biologically active substances including: saponins, flavanoids, alkaloids, phytosteroids and glycosides[3].

The aim of the present study was to determine the effect of Tribulus terrestris on Leydig cells using prepubertal rats as an experimental animal as Tribulus terrestris, is suggested to be effective in treatment of sexual dysfunctions by increasing serum LH and testosterone[6].

MATERIALS AND METHODS

Female and male adult albino rats were housed in experimental research laboratory of University of Health Sciences. One male and three female rats were housed together in a single cage for mating. Vaginal plug was observed in the morning after keeping the female and male rats together to confirm pregnancy[7]. Rats were monitored at 8-hour interval to observe the time they deliver[8]. After 21 days neonates were born; they were kept with their

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mothers and observed for any congenital anomaly. Each of the 20 male neonates so obtained was given identification mark and divided randomly were into two groups of 10 pups each (A: control and B: experimental). Each group was kept at controlled room temperature (22±2°C) and humidity of 55±10%.[7] Rats were weaned to rat chow and water ad libitum after being fed on mother's milk.

Experimental Group: Received Tribulus terrestris extract in a single oral dose of 70 mg/kg[9] daily for 20 days starting at 2 weeks of age.

Control Group: Received equal amount of weight related distilled water for the same duration.

Each animal was sacrificed on day 34 post-natally. The animal after being fully anesthetized; was laid in a supine position: its extremities were fixed to dissecting board with the help of adhesive tape. Dissection was started after ensuring complete aseptic techniques. Animal fur was soaked with ethanol to prevent contamination. Instruments were completely cleaned and Bouin's fixative[10,11] was prepared before the start of experiment. A vertical midline skin incision was made from xiphoid to pubic symphysis; it was extended laterally by a transverse incision on each side. Testes were moved out of the body cavity by pulling the tail of the epididymides. The blood vessel and vas deferens were severed allowing removal of testes and epididymides. Soon after its removal testes was separated from epididymides.[12] The right testis of each animal was sectioned transversely along the midline,[13] and immersed immediately in Bouin's fixative [10,11] for 24 - 48 hours. The tissue was embedded in paraffin and blocks were prepared in a usual way. By using a microtome 4 µm thick sections were obtained. The sections were stained with H & E and PAS and examined with a light microscope at different magnifications.

Counting the number of Leydig cells per interstitial space of testis: Spherical and ovoid Leydig cells per interstitial space[14,15] were counted using Leica 1000 DM microscope and X63 objective lens. Leydig cells were counted from 150 interstitial spaces randomly selected from each group of animals. One H & E stained section from each of the ten animals of the group was examined and total of three hundred observations made.

Statistical analysis: The statistical analysis was carried out using computer software Statistical Package for Social Sciences (SPSS) version 18.0. The arithmetic mean of observations and standard error of mean values were calculated. By the independent-samples t test in SPSS, the significance between two means was calculated. The difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS

All animals of Experimental and Control groups at the time of taking blood sample were healthy; there was no morbidity or mortality among the groups; their suckling/feeding behaviour was normal and showed no sign of any ailment. In the control group animals, number of Leydig cells per interstitial space of testes varied from 6.00 to 8.00 with Mean and SEM, 7.10 ± 0.23; in the treated group it ranged from 7.00 to 11.00 with Mean and SEM, 8.6±0.49. Difference in the mean number of Leydig cells per interstitial space of testes was statistically significant p<0.014 (Table 1).

Table 1: Comparison of mean number of Leydig Cells per interstitial space of testes of animals among experimental and control groups after therapy.

Parameter	Control Group A (10) Mean±SEM	Experimental Group B (10) Mean ± SEM	p-value
Leydig Cell count per interstitial space of testes	7.10 ± 0.23	8.6 ± 0.49	0.014*

P value: 0.014*

Figure in parenthesis indicate total number of animals in each group.
*Control group Vs Experimental group: p-value<0.014

Fig.1: Mean number of Leydig Cells per Interstitial Space of Testes of Animals of Control and Experimental Groups after Therapy.

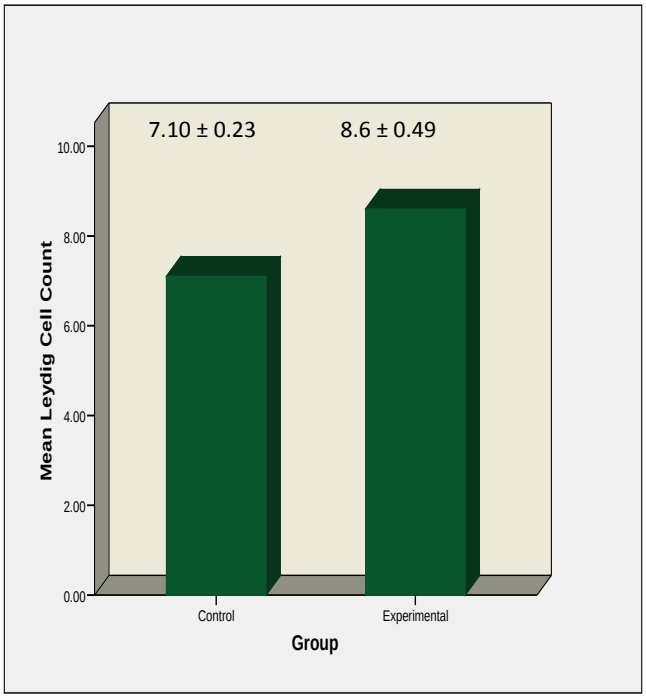


Fig.2. Photomicrograph of testis from group A illustrating seminiferous tubule which contains spermatogonia (yellow arrow) and primary spermatocytes (green arrow). The adjacent seminiferous tubule contains a spermatid (black arrow). Interstitial cells of Leydig (blue arrow) are conspicuous within the intertubular tissue. H&E stain. X400.

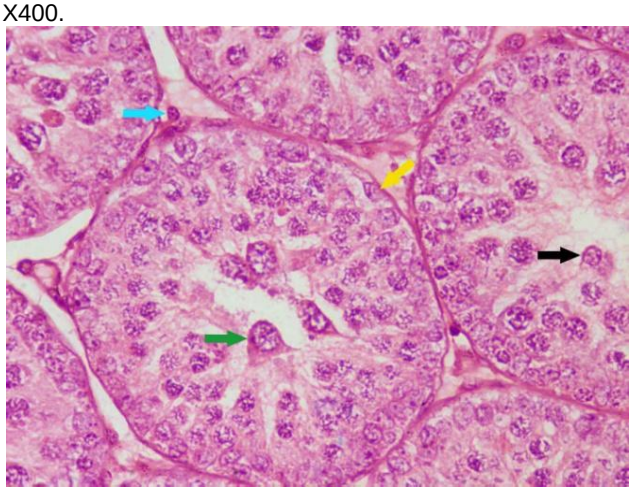
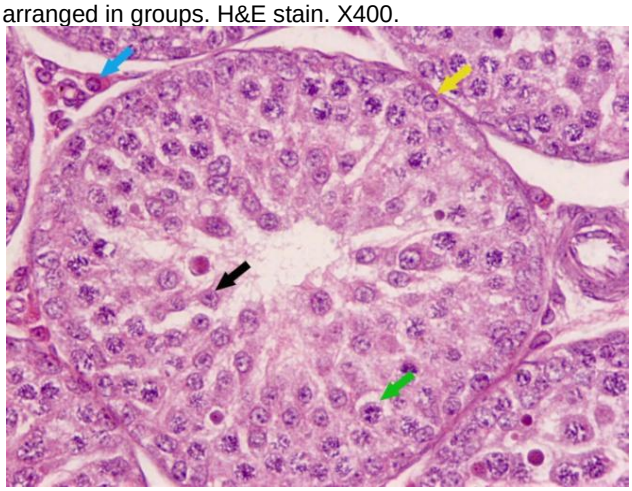


Fig.3. Photomicrograph of testis from group B illustrating seminiferous tubule which contains spermatogonia (yellow arrow), primary spermatocytes (green arrow) and rounded spermatids (black arrow). Leydig cells (blue arrow) are arranged in groups. H&E stain. X400.



DISCUSSION

Tribulus terrestris, one such herb, has been used for centuries as a therapy for various diseases affecting liver, kidney, cardiovascular and immune systems.[16] There is not much literature or data available regarding its effect on Leydig cells of prepubertal animals, therefore, we conducted this study on prepubertal rats to assess its effect on the Leydig cells as Leydig cells produce testosterone which is the major male reproductive hormone.

The experimental animals had normal food and water intake and were active without any sign of ill health; there was no mortality seen in either of the two groups; the experimental animals weighed more than those of the controls indicating that Tribulus terrestris administration did not have any negative effect on the survival of experimental animals. These findings agree with the observations made by Çek. Ş et al (2007)[17] who showed that after Tribulus terrestris treatment of Convict Cichlid (Cichlasoma nigrofasciatum) the survival ratios at the termination of the experiment in the controls were similar to those observed in the Tribulus terrestris treated group.

Mean number of Leydig cells per interstitial space of testes of animals in the experimental group was greater than that in the control ($p<0.014$). 20 days after the Tribulus terrestris treatment the mean number of Leydig cells per interstitial space of testes was 8.6±0.49, whereas, control group had a mean, 7.10±0.23. Leydig cells were seen scattered within the intertubular tissue of control group, whereas, they were arranged in the form of several groups in experimental animals. The suggested mechanism of action of Tribulus terrestris could have possibly enhanced differentiation of interstitial cells of Leydig cell. Benton L et al (1995)[18] suggested that LH and androgens act together to stimulate conversion of spindle-shaped Leydig cell progenitors into spherical or oval differentiated Leydig cells.

This has been documented in studies that high concentration of intratesticular testosterone due to Leydig cell tumor produced precocious puberty in prepubertal boys.[19,20] Thus increased number of Leydig cells in our study may lead to increased testosterone which in turn may result in precocious puberty.

CONCLUSION

The results of this study conclude that Tribulus terrestris has a stimulating effect on Leydig cells which may have led to increased testosterone levels producing precocious development of the testes. This needs further investigations, wherein the titer of testosterone, both in serum and testes, and LH in serum are also determined in an experimental setup using Tribulus terrestris.

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