ORIGINAL ARTICLE

Effects of Tribulus Terrestris on Sertoli Cells of Prepubertal Albino Rats

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ABSTRACT

Spermatogenesis depends on various hormones which include androgens. Androgens are essential for the multiplication and maturation of germ cells and also for somatic cells including Sertoli cells. Sertoli cells have FSH and androgen receptors; FSH and androgens act on Sertoli cells to produce factors which are essential for the development of germ cells. Tribulus terrestris is suggested to increase testosterone and in the Sertoli cells testosterone mediates androgen receptor transcription and also increases intracellular calcium concentration. Thus, the objective of this study was to determine the effect of Tribulus terrestris on Sertoli cells using prepubertal rats as an experimental animal. Male rat pups were divided randomly into two groups consisting of 10 rats each. Group 2 served as experimental and was given Tribulus Terrestris extract at a dose of 70mg/kg orally. Administration of Tribulus terrestris extract was started when pups were 14 days old and continued for 20 days. Group 1 served as control and was administered with equal amounts of distilled water. After sacrificing the pups, their testes were taken out of the body cavity, fixed, processed and then embedded in paraffin. 4 micrometer thick sections were then made using microtome. Sertoli cells were counted in cross sections of seminiferous tubules under X400 magnification. Experimental animals showed statistically significant increase in number of Sertoli cells as compared to Control group.

Keywords: Spermatogenesis, Sertoli cells, Testes

INTRODUCTION

Spermatogenesis is a biological process of gradual transformation of germ cells into spermatozoa within seminiferous tubules of the testis. This process involves stem cell multiplication and differentiation, genome reorganization during meiosis, and a very specific cyto-differentiation to produce highly specialized sperm cells[1]. Spermatogenesis depends on various hormones which include gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), Luteinizing hormone (LH) and androgens. These hormones are essential for the multiplication and maturation of germ cells and also for somatic cells including Sertoli and Leydig cells. Sertoli cells are found within the seminiferous tubules and they provide nutrition to the developing germ cells. Sertoli cells have FSH and androgen receptors; FSH and androgens act on Sertoli cells to produce factors which are essential for the development of germ cells[2,3,4]. Number of spermatogonia, spermatocytes, spermatids and spermatozoa and size of the testis is dependent upon number of Sertoli cells[5]. Adjoining Sertoli cells also form junctions which contribute to the formation of blood testes barrier. Germ cells when cross blood testes barrier become reliant on Sertoli cells for growth and nutrition[6].

Herbalism more or less disappeared from therapeutic map about a century ago in the western world and the modern synthetic pharmaceutical preparations have been in vogue for long. However, many drugs currently being used for sexual and erectile dysfunctions are based on active principles derived from the plants[7]. Tribulus terrestris is one such traditional herb which is used for various purposes in folk medicine. It has been found to dilate the coronary arteries and improve cardiac circulation and thus is used in treating angina pectoris[8]. It triggers melanocyte proliferation in the treatment of vitiligo[9] and has cytotoxic activity.[10] It is also reported to have a protective effect on genetic damage[8] and reduces the amount of urinary oxalate in rats.[11] The activity of the plant against both gram-positive and gram-negative bacteria reveals the presence of broad spectrum antibiotic compounds in the plant[12].

The objective of this study was to determine the effect of Tribulus terrestris on Sertoli cells using prepubertal rats as an experimental animal as Tribulus terrestris is suggested to increase testosterone[13] and in the Sertoli cells testosterone mediates androgen receptor transcription and also increases intracellular calcium concentration[14] and it is implied that Sertoli cells under the effect of

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testosterone produce factors which are responsible for maturation of germ cells[15].

MATERIAL AND METHODS

Three female and one male adult albino rats were kept together in a cage for mating. Pregnancy was confirmed by observing vaginal plug in the morning.[16] Pregnant female rats were then checked at regular intervals. After twenty one days neonates were born and were fed on mother's milk. Pups were then slowly weaned to rat chow and water ad libidum and then separated from their mothers. Male pups were identified and divided randomly into two groups consisting of 10 rats each. Only those pups were included in this study which were healthy and above 20 grams of weight. Each group was kept at controlled room temperature (22±2°C) and humidity of 55±10%.[16] Group 2 served as experimental and was given Tribulus Terrestris extract at a dose of 70 mg/kg orally. Administration of Tribulus terrestris extract was started when pups were 14 days old and continued for 20 days. Group 1 served as control and was administered with equal amounts of distilled water.

After administration of Tribulus terrestris extract to experimental group and distilled water to control group for 20 days, animals were sacrificed. The animals were completely anesthetized; they were laid flat on the dissecting board. To avoid contamination their furs were drenched in ethanol and their limbs were fixed using tape. A vertical abdominal incision starting from xiphoid process of sternum and ending at pubic symphysis was made. Abdominal cavity was opened and testes were removed from the cavity by pulling epididymides. Ductus deferens and epididymides were separated from the testes.[17] Each testis was cut transversely along the midline[18] and immersed separately in freshly prepared Bouin's fixative[19,20] for one to two days. Testes were then processed, embedded in paraffin[21] and 4 micrometer thick sections were made. Sections were then stained with hematoxylin and eosin and examined under light microscope.

Sertoli cells were counted in cross sections of seminiferous tubulues under X400 magnification. Sertoli cells were counted in 30 tubules from each animal and thus total of 600 observations were made from both the groups. Only those Sertoli cells were counted which had prominent nucleolus and had classic morphological features[22,23].

Statistical analysis was done by using SPSS version 20. Mean of observations from each group and standard error of mean was calculated and then means of the two groups were compared by using independent samples t test. Difference was considered statistically significant if p value was less than 0.05.

RESULTS

Animals from both the groups were healthy at the end of the experiment and there were no signs of any illness. Their feeding behavior was normal. There was no mortality during the experiment. Spermatogonia, spermatocytes and spermatids were arranged in concentric layers within the seminiferous tubules. Ovoid nuclei of Sertoli cells with prominent darkly stained nucleolus were visible in the basal region. In the control group, the mean and standard error of mean of Sertoli cells per tubule was 8.24±0.22, whereas, in the experimental group it was 9.47 ±1.47. Difference in the mean of Sertoli cells of control and experimental groups was statistically significant i.e., $p<0.05$ (Table 1).

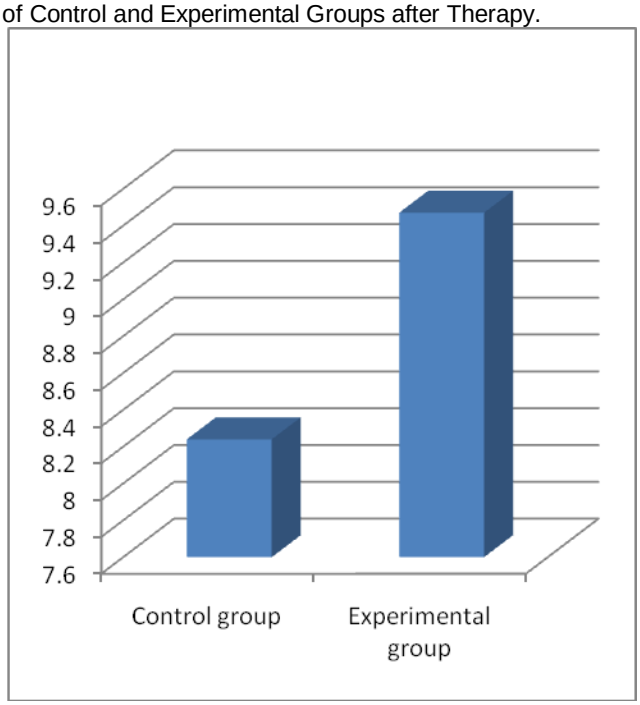
Table 1: Comparison of mean number of Sertoli Cells of animals among experimental and control groups

Parameter	Control Group (10) Mean±SEM	Experimental Group (10) Mean ± SEM
Sertoli cell count per tubule	8.24 ± 0.22	9.47 ± 1.47

P value: 0.05

Fig. Parenthesis indicate total number of animals in each group. *Control group versus Experimental group: p-value<0.05

Fig.1: Mean number of Sertoli Cells per tubule of Animals of Control and Experimental Groups after Therapy.



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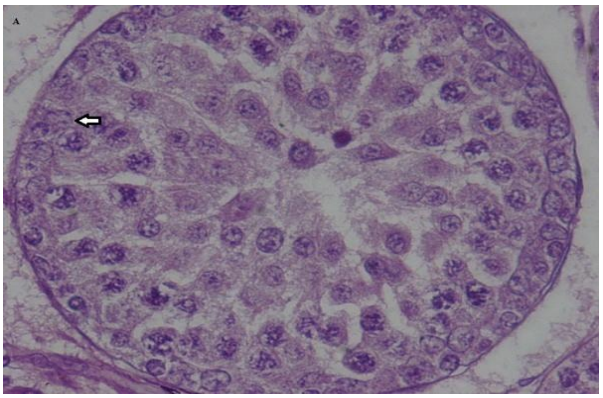

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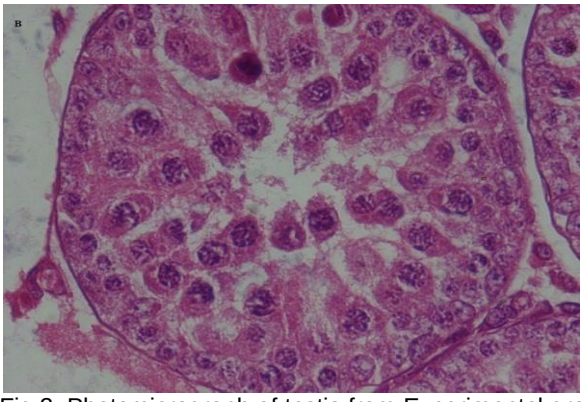

Fig.2: Photomicrograph of testis from Experimental group II (A) depicting increased oval shaped nuclei of Sertoli cells (Arrow) with darkly stained nucleoli as compared to Control group I (B). H&E stain. X400.

DISCUSSION

Our observations on experimental group revealed that administration of Tribulus terrestris increased Sertoli cell number in prepubertal rats. These findings are consistent with a study which showed that in infant male lambs number of Sertoli cells increased when exposed to increased testosterone levels prenatally[24] and Tribulus terrestris is suggested to increase LH levels leading to enhanced testosterone secretion from Leydig cells, which in turn stimulates Sertoli cells[13].

Our findings also agree with another study in which immature monkeys, Macaca Mulatta, of 12 to 18 months of age when administered with either follicle stimulating hormone or testosterone or a combination of both showed statistically significant increase in the number of Sertoli cells within the seminiferous tubules[25].

Another study also showed that Tribulus terrestris administration led to protective effect on Sertoli cells from cadmium induced damage. The author attributed Sertoli cell protection to stimulating effect of Tribulus terrestris to increase testosterone production[26].

According to one study Tribulus terrestris when given orally to male mice for 2 weeks led to an increase in the number of Leydig cells[27] This increase in Leydig cell number may have led to enhanced testosterone production resulting in increased Sertoli cell number in our study.

CONCLUSION

Our study has shown that administration of Tribulus terrestris has stimulating effect on Sertoli cells of prepubertal rats which may be due to increased testosterone levels. This requires further investigation in which serum testosterone and FSH levels are also determined after adminisring Tribulus terrestris extract.

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