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EFFECTS OF TRIBULUS TERRESTRIS ON TESTICULAR DEVELOPMENT OF IMMATURE ALBINO RATS

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ABSTRACT

The present study was designed to investigate the effects of extract of Tribulus terrestris on body weight and testicular development of prepubertal rats. The study was conducted in the Department of Anatomy, University of Health Sciences, Lahore. Two-week old rats were divided into two groups of 10 pups each (A: control and B: experimental). Group B was given Tribulus Terrestris in an oral dose of 70mg/kg daily for 20 days. Pups were weighed and sacrificed on 34th day post-natally; their testes were removed for gross and microscopic studies using 4 µm thick H & E and PAS stained histological sections. Statistical analysis was done using independent-samples t test. Pups received tribulus terrestris extract showed statistically significant increase in mean body ($p<0.05$) and paired testes weight ($p<0.05$) without significant effect on the mean relative tissue body weight index ($p>0.05$). Histological slides of the testes showed a significant increase in seminiferous tubules containing early spermatids in the treated group when compared to that of control ($p<0.05$). The mean diameter of seminiferous tubules in the treated group was also noticeably increased ($p<0.05$). Spermatids of the experimental group were at acrosomal phase of spermogenesis, whereas, those of control group were at Golgi phase, implying thereby that spermatogenesis was present at an advanced stage in the experimental as compared to the control group of animals.

Key words: *Testis, Rat, Prepubertal, Tribulus Terrestris.*

INTRODUCTION

Procreation is essential for the propagation of race. Infertility is defined as the inability to conceive after 12 months of sexual practice without the use of contraception.[1,2] Approximately 15% of couples attempting their first pregnancy meet with failure and, in nearly 50% of all infertility cases, the cause is attributed to the male.[3] Although there has been considerable improvement in the understanding and treatment of female infertility, practically little advancement is seen in dealing with this problem in the male.[4]

Tribulus terrestris is a herbal remedy which is used for various purposes in folk medicine. It has been used as tonic, aphrodisiac, astringent, analgesic, stomachic, anti-hypertensive, diuretic and urinary anti-septic.[5]

Tribulus terrestris is a flowering plant of the family Zygophyllaceae. It is about 30 to 70cm high; it grows as a summer annual, has pinnately compound leaves, yellow flowers and stellate shaped carpel fruits.[6]

Traditional herbs have emerged in the past few years as an 'instant' treatment for sexual and erectile dysfunctions.[7] *Tribulus terrestris*, is suggested to be effective in treatment of such dysfunctions by increasing serum LH and testosterone,[1] and conversion of its phytochemical derivative, protodioscine to dehydroepiandrosterone (DHEA).[7] The chemical structure of protodioscin is very similar to that of DHEA.[8]

A study conducted on males, with moderate idiopathic oligozoospermia, showed that protodioscin treatment of men resulted in increased level of testosterone.[1] Marshall[9] showed that administration of exogenous testosterone initiates spermatogenesis in an immature nonhuman primate, Macaca fascicularis with an increase in testicular volume & testicular testosterone concentrations. Spermatogenesis at different stages of development was found in testes of monkeys treated with testosterone; the sperms were also found in the ejaculates of a couple of immature monkeys. This has been documented in studies that high concentration of intratesticular testosterone, due to Leydig cell tumor, produced precocious puberty, in prepubertal boys.[10,11] Patients with delayed puberty benefit from testosterone therapy enabling them to reach adult body composition.[12] Udagawa[13] showed that testosterone administration enhanced the regeneration of chemically impaired spermatogenesis in rats.

Contrary to the above findings, in 2005, Neychev and Mitev[14] reported that *Tribulus terrestris* did not induce an increase in testosterone or LH in young men and Brown[15] (2000) found that a com-

mercial supplement containing androstenedione and herbal extracts including *Tribulus terrestris*, was not effective in raising serum concentrations of free or total testosterone.

The present study is, therefore, designed to examine the effects of *Tribulus terrestris* on spermatogenesis using prepubertal rats as an experimental model with the hope that the results of this study may pave the way for treating delayed puberty and male infertility.

MATERIALS AND METHODS

Six female and two male adult albino rats were procured from National Institute of Health, Islamabad and were kept for two weeks in experimental research laboratory of University of Health Sciences to acclimatize them. One male and three female rats were housed together in a single cage for mating. Neonates born after 21 days were kept with their mothers and examined for any congenital anomaly. Each of the twenty male neonates so obtained were divided 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%.[16] All pups were fed on mother's milk and gradually weaned to normal rat chow and water ad libitum. The pups with 20 grams of weight were included in the study. The experimental group was given *Tribulus terrestris* extract in a single oral dose of 70 mg/kg daily for 20 days, starting at 2 weeks of age, whereas the control group was given equal amount of weight related distilled water for the same duration. At the end of the experimental period, each animal was weighed and sacrificed on day 34 postnatally.

A vertical midline skin incision was given; abdomen was opened and the retractable testes were removed after severing the blood vessels, vas deferens and epididymides.[17] Weight of paired testes from each animal was recorded in grams. The right testis of each animal was sectioned along the midline[18] and immersed in Bouin's fixative[13,19] for 24 hours. The tissue was embedded in paraffin and blocks were prepared in a usual way; 4 µm thick sections were obtained by using a microtome (Shandon Finesse ME +). These were stained with H & E and PAS and examined with a light microscope at different magnifications.

Spermatogenesis was assessed by a method which depended upon scoring 'cross sectional' profiles of seminiferous tubules according to Johnsen's criteria.[20-22] The criteria use scores from 1 to 10 and are as follows: 10. Complete spermatogenesis with many spermatozoa. 9. Many spermatozoa present but germinal epithelium disorganized with marked sloughing or obliteration of lumen. 8. Only a few (<5) spermatozoa. 7. No spermatozoa but many spermatids. 6. No spermatozoa and only a few spermatids (<5). 5. No spermatozoa or spermatids but many spermatocytes. 4. No spermatozoa or spermatids and only a few spermatocytes (<5). 3. Spermatogonia are the only germ cells present. 2. No germ cells but sertoli cells are present. 1. No cells in tubular section.

Diameter of seminiferous tubules was measured using Leica 1000 DM microscope and X10 objective lens with ocular micrometer.

STATISTICAL ANALYSIS

The statistical analysis was carried out using computer software Statistical Package for Social Sciences (SPSS) version 15.0. The arithmatic mean of observations and standard error of mean values were calculated: the significance between two means was calculated by the independent-samples t test in SPSS. 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 sacrifice were active and healthy.

Body Weight

The mean body weight of animals in the treated group was significantly increased as compared to control group: $p<0.05$ (Fig. 2).

The scrotal sacs were of normal shape, color and rugosity with freely hanging testes in both groups. The testes presented pinkish white hue, were soft in consistency, richly supplied with blood vessels and showed stringing out phenomenon, in both control as well as treated groups, when put in fixative.

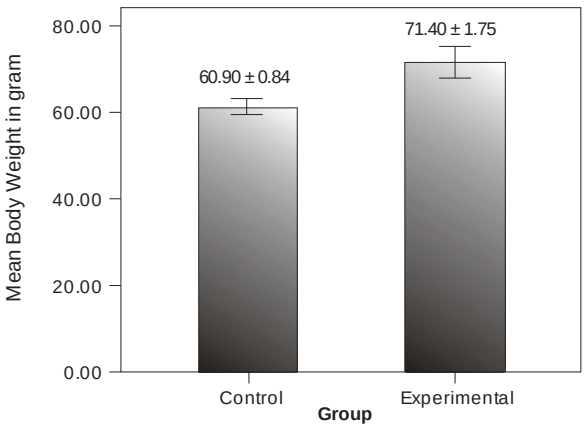

Fig. 2: *Mean body weight (g) of animals of control and experimental groups.*

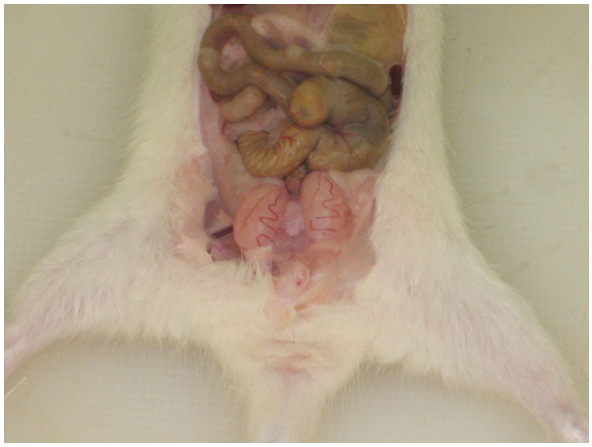

Fig. 1: *Photograph of a rat showing the technique to remove testes.*

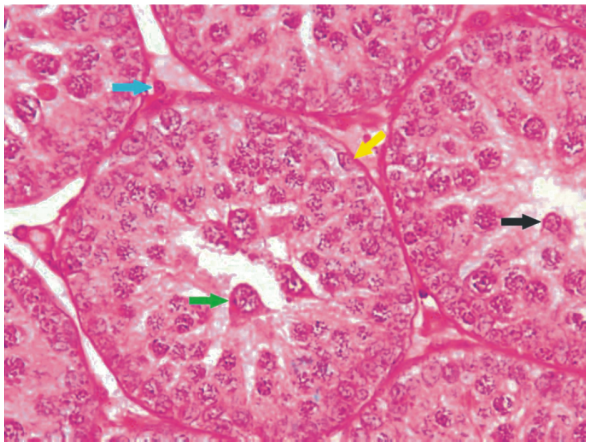

Fig. 5: Photomicrograph of testis from group A illustrating seminiferous tubule which contains spermatogonia (yellow arrow) and spermatocytes (green arrow). The adjacent seminiferous tubule contains a spermatid (black arrow). Interstitial cells of Leydig (blue arrow) are conspicuous within the intertubular tissue. H&E stain. X400.

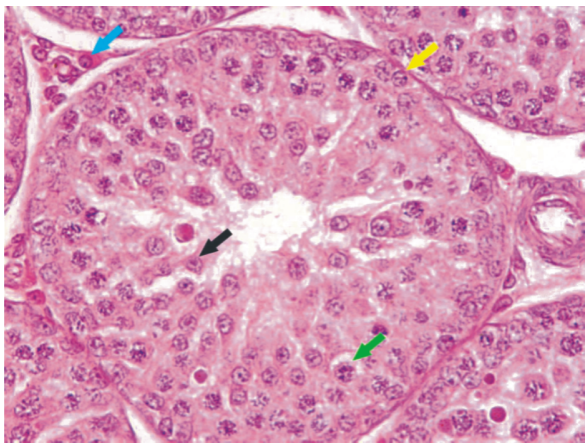

Fig. 6: Photomicrograph of testis from group B illustrating seminiferous tubule which contains spermatogonia (yellow arrow), spermatocytes (green arrow) and rounded spermatids (black arrow). The number of spermatids has noticeably increased when compared with those from group A, Leydig cells (blue arrow) are arranged in groups. H&E stain. X400.

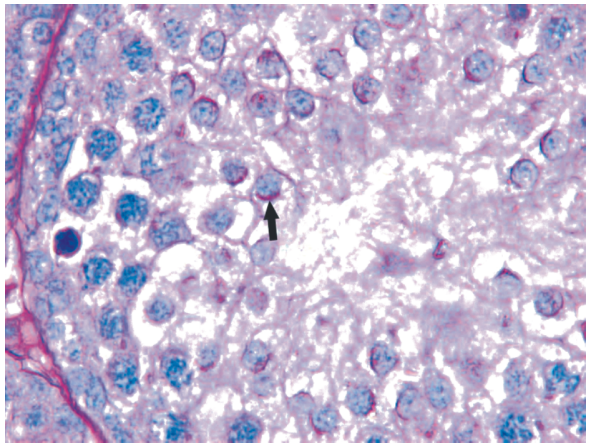

Fig. 7: *Photomicrograph of testis from group B showing spermatids during acrosomal phase of spermiogenesis (black arrow). PAS stain. X630.*

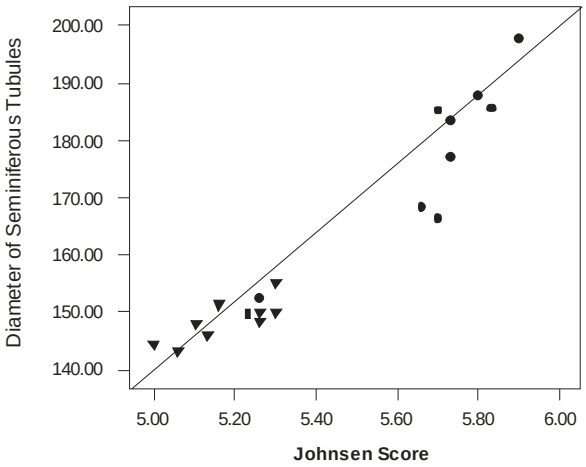

Fig. 10: *Scatter plot of Johnsen Scores Vs Diameter of Seminiferous Tubules (μ) of Experimental ● and Control ▼ Groups ▮ shows overlapping of experimental and control group readings.*

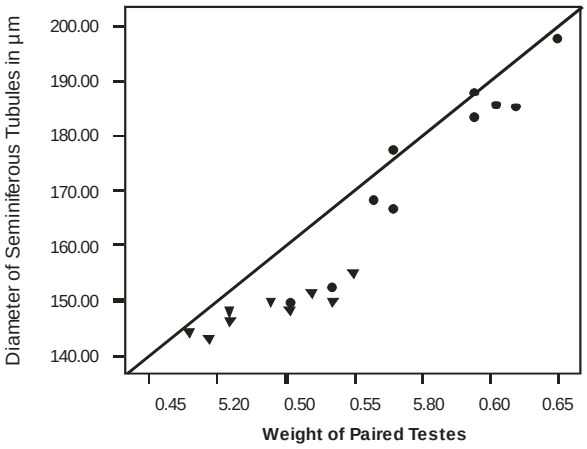

Fig. 11: Scatter plot of Weight of Paired Testes (g) Vs Diameter of Seminiferous Tubules (μ) of Experimental ● and Control ▼ Groups.

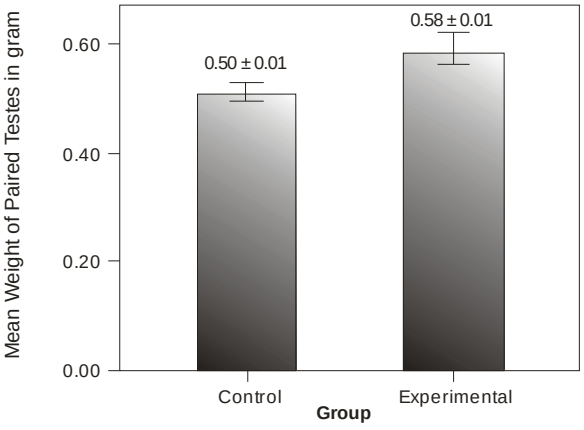

Fig. 3: *Mean weight of paired testes (g) of animals of control and experimental groups.*

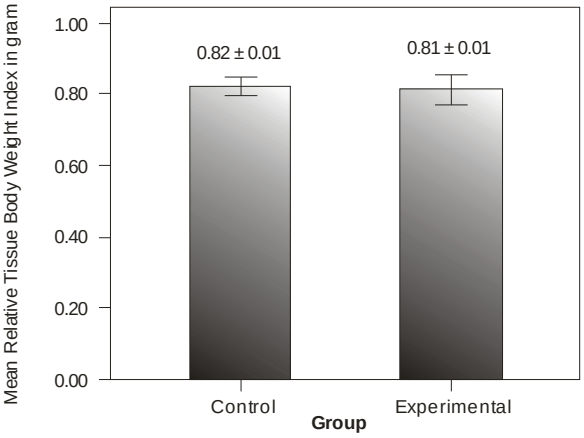

Fig. 4: *Mean relative tissue body weight indices of animals of control and experimental groups.*

Weight of Paired Testes:
Increase in the weight of paired testes in treated group compared with the control group was statistically significant: $p<0.05$ (Fig. 3).

Relative Tissue Body Weight Index:
Difference in the relative tissue body weight indices of animals in A and B groups was not statistically significant: $p>0.05$ (Fig.4).

Histological Observations of Testes:
Light microscopy revealed that the seminiferous tubules sectioned in various profiles in both control and experimental groups when stained with PAS showed regular and thin basement membrane. Lumen was observed to be better developed in experimental than in the control group.

Sections when stained with H & E showed cells of spermatogenic lineage- spermatogonia, spermatocytes and rounded spermatids, stacked in several layers in seminiferous tubules of control and experimental groups. Seminiferous tubules containing rounded spermatids were noticeably increased in the treated group (Figs. 5 & 6). Few elongated spermatids were seen in some of the seminiferous tubules of the treated group.

PAS positive Golgi zone of rounded spermatids was seen in seminiferous tubules of control group depicting Golgi phase of spermiogenesis. Formation of acrosomal cap over the anterior half of nucleus of spermatids was visible in PAS stained tubules of the treated group only (Fig. 7), indicating acrosomal phase of spermiogenesis, implying thereby that spermatogenesis was present at an advanced stage in the experimental as compared to the control group of animals.

Johnsen Score:
Mean Johsen score was 5.65 ± 0.07 in group B, whereas in group A, it was 5.18 ± 0.03. Difference in mean between the two groups was statistically significant: $p<0.05$ (Fig. 8).

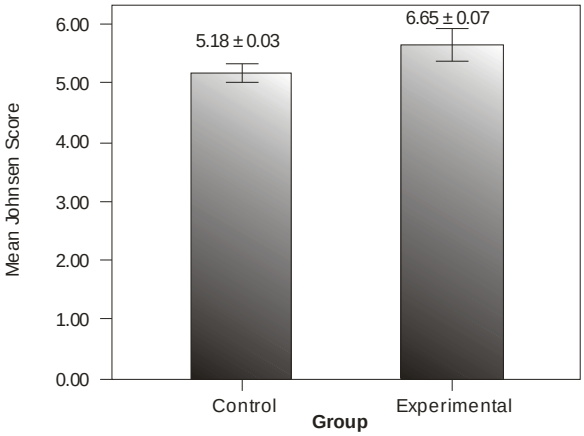

Fig. 8: *Mean Johnsen Scores of Animals of Control and Experimental Groups.*

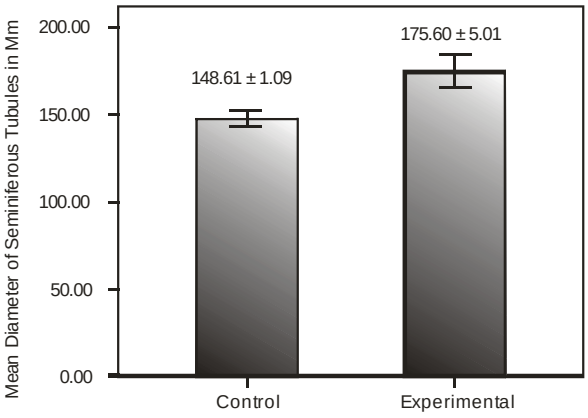

Fig. 9: *Mean Diameter of Seminiferous Tubules (μ) of Animals of Control and Experimental Groups.*

Diameter of Seminiferous Tubules:
Mean diameter of seminiferous tubuls was 175.60 ± 5.01µm in group B whereas in group A, it was 148.61 ± 1.09µm. The difference between the two groups when compared was statistically significant p-value < 0.05 (Fig. 9).

A significantly positive correlation (r = 0.959) was found when Johnsen scores were plotted against diameter of seminiferous tubules of both control and experimental groups (Fig. 10).

A significantly positive correlation (r = 0.958) was found when weight of paired testes were plotted against diameter of seminiferous tubules of both control and experimental groups (Fig. 11).

DISCUSSION

Our observations on experimental group revealed that *Tribulus terrestris* administration resulted in increased body and testicular weight which were statistically significant when compared with the control. These findings agree with the findings of Gauthaman[23] who showed that treatment of castrated rats with *Tribulus terrestris* extract resulted in increased body and prostate weight; Gauthaman,[24] while evaluating sexual effects of different doses of *Tribulus terrestris* on rats, observed a significant increase in body weight of treated rats which he presumed was due to androgenic effect of *Tribulus terrestris*, producing a stimulus to increase the appetite. Androgens have a major role in the growth and differentiation of many tissues in addition to the organs of reproductions; Androgens are also responsible for the pubertal development of the testes.[25]

Our findings also agree with the findings of Çek[26] who showed that the *Tribulus terrestris* treatment siginificantly improved the growth rate of Convict Cichlid (Cichlasoma nigrofasciatum), total body length and weight were significantly increased in the experimental fish as compared to the controls. They also showed that males in the control group had smaller gonads than the experimental group.

Our findings showed that average diameter of seminiferous tubules in the experimental group was greater than that in the control. Relative increase in diameter of seminiferous tubules is reported to be indicative of the androgenic effect of *Tribulus terrestris* on experimental animals; increased surface area of the basement membrane is considered to be associated with higher testosterone production. Seminiferous tubule diameter was taken as an indirect measure of increase in spermatogenic function since surface area of spermatogenic epithelium anchors more Sertoli cells and spermatogonia.[19]

Johnsen's criteria offer a convenient and rapid method for registration of spermatogenesis.[20] The increase in the Johnsen's score of the treated group when compared with the control group was statistically significant. Johnsen[20] showed a high correlation between log total sperm count and the mean Johnsen score. Thus an increase in the mean Johnsen score in our study is an indirect evidence of improvement in the sperm count. These findings agree with the findings of Arsyad[1] who showed that *Tribulus terrestris* (protodioscin) treatment led to an invariable increase in concentration of spermatozoa in humans to approximately 160%. The author attributed this to an increase in the LH level which acted on Leydig cells and enhanced testosterone secretion, and stimulated Sertoli and germinal cells.

Viktorov[27] reported that administration of *Tribulus terrestris* preparation significantly increased the number of spermatogonia, spermatocytes and spermatids in the testes of adult rats. They suggested an intensified DNA synthesis under the effect of protodioscin.

Çek[26] showed that spermatogenesis was accelerated among the *Tribulus terrestris* treated group of Cichlasoma nigrofasciatum compared to the control group; further, it was observed that the histological response of the testes in *Tribulus terrestris* treated groups invariably included an increased number of spermatogenetic cysts and an excess of late stages of spermatogenesis.

In our study significantly positive correlation was found when Johnsen scores were plotted against diameter of seminiferous tubules. As measurement of tubular diameter is a significant indicator of testicular function[20] an increase in the mean Johnsen score in our study is an indirect evidence of improvement in the sperm count as well as the testicular function.

Comparison of weight of paired testes with diameter of seminiferous tubules showed a highly significant relationship, the more active testes were considerably heavier and had larger diameter of seminiferous tubules.

The results of this study **conclude** that *Tribulus terrestris* has a complex stimulating effect on germinative and endocrine functions of the testes producing its precocious development. 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*. Further, a detailed investigation of intracellular nucleotides is required for a better understanding of the mechanism of action and clinical usefulness of *Tribulus terrestris* in treating the cases of male infertility and delayed puberty.

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