Immunohistochemical Study of Leydig Cells in the Testicular Interstitial Tissue of Rats Treated with Tribulus Terrestris Using P450scc.

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ABSTRACT:

BACKGROUND:

Tribulus terrestris has been commonly used in folk medicine to energize, vitalize and improve sexual function and physical performance in men and laboratory rats.

OBJECTIVE:

To study the effect of *Tribulus terrestris* on the number of Leydig cells.

MATERIALS AND METHODS:

Tribulus terrestris was given to mature male rats as an oral single herbal suspension in a dose of 2.0mg /1000gbody weight for 14 days to stimulate spermatogenesis. Formalin fixed paraffin-embedded tissue sections were performed for histological, immunohistochemical and morphometrical studies.

RESULTS:

Histological study revealed wider seminiferous tubules and increased spermatocytes population with an increased sperm density inside the lumen of the tubules. Morphometrically, the diameters of seminiferous tubules and thickness of the germinal epithelia were significantly increased in *Tribulus terrestris* treated rats than that of the control group. There was no significant difference between the number of Leydig cells in the control and experimental groups.

CONCLUSION:

The activity of Leydig cells, manifested by the increments in the diameters, thickness of germinal epithelia and the density of the sperms inside seminiferous tubules, was increased but their number remain unaffected in spite of using the aphrodisiac agent, *Tribulus terrestris*.

KEY WORDS: rat testis, tribulus terrestris, leydig cells.

INTRODUCTION:

The mammalian reproductive axis is coordinated by the hypothalamic secretion and trophic effects of gonadotrophin releasing hormone, which is in turn controlled by negative feedback from gonadal steroids[(1)]. Leydig cells, which are steroid secreting cells, produce testosterone by enzymes present in the mitochondria and smooth endoplasmic reticulum in their cytoplasm [(2)].Approximately 8mg of testosterone is produced daily, the major source (95%) being the interstitial cells of Leydig while the remaining (5%) secreted by the adrenal glands [(3)].The plant *Tribulus terrestris* has been commonly used in folk medicine to energize, vitalize and improve sexual function and physical performance in men [(4)]. In addition it has been extensively used both in Chinese and Indian traditional medicine for the treatment of various diseases such as urinary, cardiovascular, and gastrointestinal disorders [(5,6,7)]. The medical effects of *Tribulus terrestris* are due to a number of its active phytochemicals among which are: Steroidal saponins (dioscin, protodioscin, and diosgenin) and Sterol such as β-sitosterols and stigmasterols [(4)].The aphrodisiac properties of *Tribulus terrestris* are related to the protodioscin component of the herb which is a steroidal saponin that forms 45% of the herb extract [(1)].

MATERIALS AND METHODS:

Twenty four adult (8-12 weeks of age), sexually mature, Norway Albino male rats were used in this study. These animals were housed individually in separate cages in the animal house of Baghdad medical school under normal diurnal lighting conditions, kept at a relatively controlled temperature of about 25°C, and have free access to

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water and food [8]. The sample was divided into two groups, each composed of twelve rats. The first group was the control group and the second was the experimental group. The control group received no herbal treatment while the experimental group received *Tribulus terrestris* as an oral, single daily dose, herbal suspension. A dry ripe seed of *Tribulus terrestris* was crushed in a coffee grinder, and the obtained powder was suspended in distilled water to make herbal suspension. Twenty five milligrams of *Tribulus terrestris* was suspended in fifty milliliters distilled water and given to rats in a dose of 2mg /1000 g body weights as an oral single daily dose for fourteen days [4]. The herbal suspension was delivered slowly to experimental rats using a 5.0 ml graded pipette tube. At the end of the experiments the obtained samples were fixed in 10% neutral buffered formalin for 20-24 hours at room temperature and processed for routine paraffin blocks formation and sectioned into5.0 µm thick sections using a Histoline microtome[9]. From each tissue blocks, 10 sections were collected. Five testicular sections were mounted on charged slides and stained immunohistochemically using Leydig cell P450scc antibody to identify Leydig cells. P450scc is a mitochondrial enzyme associated with the conversion of cholesterol to pregnenolone and it was provided by biorbyt Co., catalog No. orb 5936, and detected by LSAB + system-HRP detection kit which is an extremely sensitive method provided by Dako Co. code number K0679.The other five sections were mounted on ordinary slides and used for H. and E. staining. Morphometry was done to study the means and standard deviations of diameters of seminiferous tubules (two diameters of each tubule, one perpendicular to the other, were measured then the average was taken), thickness of germinal epithelia in 25.0 rounded transversely cut seminiferous tubules/ five tissue sections, & numbers of Leydig cells in 25.0 different fields of interstitial spaces/ five tissue sections by using an eyepiece micrometer, at 400X magnification power [10] & the differences between these variables in both groups.

RESULTS:

Histological study was conducted to study the general arrangement of the tissue structure in the control group and the differences in the thickness of the germinal epithelium, diameter of the seminiferous tubules and the density of the sperms in the lumen of seminiferous tubules. Testicular sections of control rats stained with H. and E. revealed normal testicular tissue histology. The seminiferous tubules, separated by the interstitial tissue, are lined by a single layer of rounded cells, spermatogonia, followed by the primary spermatocytes. The sperms appeared with heads anchored in the germinal epithelium & the tail floating in the lumen of the seminiferous tubules. Testicular sections of *Tribulus terrestris treated* rats, stained with H. & E., revealed an increase in diameter of seminiferous tubules when compared with control group, with increased thickness of germinal epithelia mainly in the primary spermatocytes population & high density of sperms inside the lumen of the seminiferous tubules (fig. 1& 2). Morphometrical study revealed that the diameters of the seminiferous tubules and thickness of the germinal epithelia were significantly increased (p-value < 0.0005 for both) in *Tribulus terrestris* treated rats than that of control group (table 1). In addition there was no significant difference between numbers of Leydig cells in the control and experimental (*Tribulus terrestris*) groups (P-value > 0.05) (table 2, fig. 3 & 4).

Table 1: Shows the differences between mean diameters of seminiferous tubules and thickness of germinal epithelia in control and experimental groups.

	Control		Experimental group		P-value
	Mean (µm)	SD	Mean (µm)	SD	0.0005
Diameter	77.2	1.104	90.01	1.41	
Thickness of germinal epi.	22.3	0.4	32.84	0.47	

Table 2: Show the differences between the numbers of Leydig cells in the control and experimental (Tribulus terrestris treated rats) groups.

	Control group		Experimental group		P-value
	Mean	SD	Mean	SD	0.05
Leydig cells	18.63	0.99	19.21	1.101	

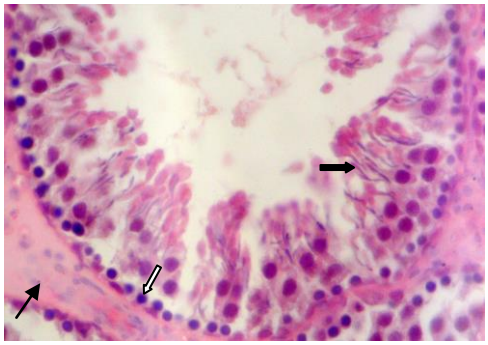

Figure 1: Shows normal histology of rat testis (control group) white arrow refers to spermatogonia, narrow black arrow refers to the interstitial tissue and wide black arrow refers to the sperms within the lumen of seminiferous tubule (magnification X 400, H. and E.).

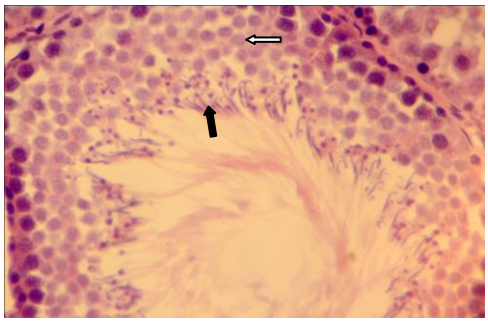

Figure 2: Shows an increment in diameter of seminiferous tubules, thickness of germinal epithelium and density of the sperms within the seminiferous tubules of experimental rats treated with *Tribulus terrestris*, white arrow refers to primary spermatocytes and black arrow refers to the sperms within the lumen of seminiferous tubule (magnification X400, H. and E.)

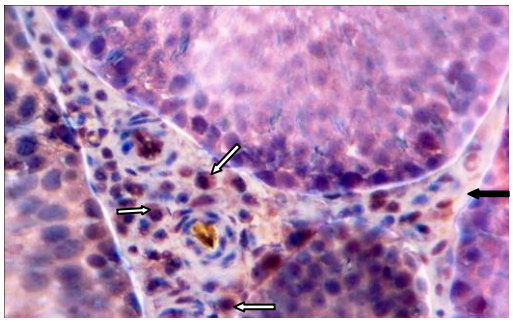

Figure 3: Shows Leydig cells within interstitial tissue of rat testis of control group, localized immunohistochemically using P450scc, white arrows referred to Leydig cells and black arrow refers to the interstitial tissue (magnification X400, haematoxylin counter stain).

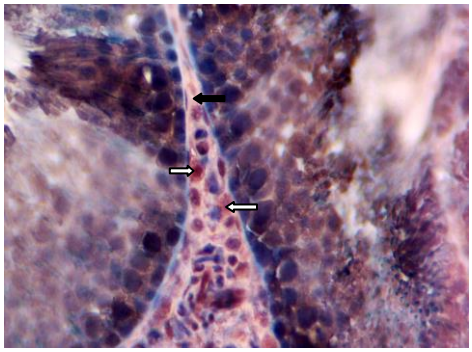

Figure 4: Shows Leydig cells within the interstitial tissue of experimental rat testis, treated with *Tribulus terrestris*, localized immunohistochemically using P450scc, white arrows referred to Leydig cells and black arrow referred to the interstitial tissue (magnification X400, haematoxylin counter stain)

DISCUSSION:

The mean diameters and thickness of the germinal epithelia of seminiferous tubules in the experimental group (*Tribulus terrestris* treated rats) were increased significantly in comparison with the control group. These results coincided with Martino-Andrade A.J., *et al.*, 2010 results in which they have reported that there was a positive effect of *Tribulus terrestris* administration on rat sperm production [(11).] In addition Gauthaman K. and Adaikan P.G., 2008 in their study found that *Tribulus terrestris* increases the sex hormones, and they attributed these results to the presence of protodioscin in the tribulus extract[(1).] Similar results were reported by Al-Yawer M., *et al.*, 2008 that *Tribulus terrestris* was responsible for the increment in androgen production from Leydig cells and thus enhanced spermatogenesis [(4)]. Gauthaman K., *et al.*, 2002 concluded that *Tribulus terrestris* extract appears to possess aphrodisiac activity probably due to androgen increasing property of its extract [(12)]. In spite of these results there was no significant difference in the mean number of Leydig cells between the control and experimental (*Tribulus terrestris* treated rats) groups. In our study the absence of the significant differences in the number of Leydig cells may not indicate the absence or diminished Leydig cells activities.

CONCLUSION:

The activity of Leydig cells were markedly increased as demonstrated by the increments in the diameters and thickness of germinal epithelium of their seminiferous tubules. However, there was no significant increase in the number of Leydig cells which were demonstrated by P450scc.

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