ORIGINAL ARTICLE

Protective Effect of Tribulus Terrestris on Nifedipine Induced Damage to Microstructure of Testes of Rats

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ABSTRACT

Objective: To investigate the protective effect of a herb *Tribulus Terrestris* on Nifedipine induced damage on microstructure of testes.

Study Design: Animal Experimental Study.

Place and Duration of Study: The present study was undertaken for a period of 18 months with effect from October, 2012 to April, 2014 at Shifa College of Medicine, Islamabad.

Materials and Methods: Male Sprague Dawley rats (N=120) were divided into four groups, each comprising of 30 rats. Group A was given Dimethyl sulfoxide (DMSO), whereas group B, C and D were given Nifedipine only, *Tribulus terrestris* only and Nifedipine and *Tribulus terrestris* both respectively, by oral route for 56 days. At the end of experiment the diameter of seminiferous tubules and height of germinal epithelium was measured in H& E stained slides of testes in all groups and then compared. Results were analyzed on SPSS version 22.

Results: There was a significant difference in mean tubular diameter and height of germinal epithelium of testes between Nifedipine only group and treated rats which were given Tribulus extract with Nefidipine, showing its protective effect on the treated group.

Conclusion*:* Extract of *Tribulus Terrestris* helps in inhibiting the damaging effects of Nifedipine on histomorophometry of testis.

Key Words: *Infertility, Testis histomorphometry, Nefidipine, Tribulus*

Introduction

Infertility, whether primary or secondary, has always been a public health concern worldwide. As many as 15% of couples have difficulty while conceiving with male factor being responsible in up to 50% of such cases.[1] Out of a long list of risk factors, certain therapeutic drugs like cytotoxic, anti-convulsant, antimalarial and antihypertensive all have been reported to induce either permanent or temporary infertility.[2] Hypertension (HTN) is an emerging problem of modern era. It has been estimated that about 1 billion people worldwide have HTN and is expected to increase to 1.56 billion by 2025.[3] National Health Survey of Pakistan assessed that only 50% of the people with HTN were diagnosed and only half of those diagnosed were getting treatment for HTN.[4] Hypertension has to be treated vigorously as it is a stepping stone of variety of complications. Numbers of antihypertensive drugs are in use, naming a few: Diuretics, Angiotensin converting enzyme inhibitors, Beta-blockers, Calcium channel blockers (CCBs). CCBs became the best-selling antihypertensive since early 1990'still today because it is effective in controlling blood pressure at all age groups.[5]

Role of Calcium ions (Ca^{2+}) as ubiquitous second messenger system has long being recognized.[6] In male reproductive organs increase in intracellular Ca^{2+} through voltage gated calcium channels (VGCCs) induced by LH is indispensable for testosterone production.Ca^{2+} ions induce increase in activity and

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transcription of Steroidogenic acute regulatory protein (StAR) which mediates the transport of Cholesterol to the mitochondria. This step is the rate-limiting step of steroidogenesis.[7] Similarly Ca^{2+} is required for the normal spermatogenesis (spermiogenesis) and sperm motility and fertilization.[8]

Out of CCBs Nifedipine is one of the most prescribed drugs in the world. Its gaining wider acceptance for treating angina pectoris, hypertension, and congestive heart failure.[9] Belonging to Dihydropyridine(DHPs) subgroup of CCBs, it acts by blocking influx of calcium ions through the L-type VGCCs of smooth muscle cells of blood vessels thus reducing the blood pressure by decreasing peripheral vascular resistance mainly at the level of small arterioles. Though all CCBs share the same mechanism of action, DHPs differ from others induration of action, rate of onset of hypotension, predictability of response and severity of adverse effects.[10] It is known in literature that therapeutic administration of CCBs has been correlated with iatrogenic reversible male infertility.[11] The available literature has stated adverse reversible effects of Nifedipine on testicular steroidogenesis and spermatogenesis.[12, 13]Therefore, any therapeutic application of CCBs must be used with vigilance in males. Any agent that may increase testosterone production and spermatogenesis can be helpful for enhancing fertility in patients taking CCBs.

Tribulus terrestris (TT) is a natural herb (a flowering plant) used for treating many diseases. It finds important position in Ayurveda for the cure of sexual dysfunction in males.[14, 15]TT fruit particularly contains two steroidal saponins Protodioscin (PTN) and Prototribestin in its extract.[16]PTN is reported to increase the levels of testosterone, DHEA and DHT[15] hence improves libido and spermatogenesis.[17] It has been proved to be effective in stimulating spermatogenesis and activity of Sertoli cell in rats.[18]
As its a known fact that Nifedipine induces infertility through changes in calcium influx through voltage gated calcium channels and Tribulus increases the production of male sex hormone promoting spermatogenesis, its protective effect on CCB induced damage on microstructure of testes should be studied.[11,12,13] As on this protective effect of Tribulus no literature is available so far. Therefore the objective of the study was to determine the protective effect of TT on Nifedipine induced changes in microstructure of seminiferous tubules.

Materials and Methods

An animal experimental type of research was undertaken from October, 2012 to April, 2014 at the end College of Medicine, Islamabad and National Institute of Health Sciences (NIH), Islamabad. After an approval from the Institutional review committee of Shifa College of Medicine. One hundred and twenty (N=120), 90-120 days old male Sprague Dawley rats, weighing approximately 180-250g, were selected through non-probability convenience sampling from the animal house of NIH, Islamabad. Rats with any obvious physical pathology were excluded. They were divided into four groups having thirty rats each and were tagged with permanent marker on their dorsal body wall in their respective groups. Each group was kept in separate cage under standard laboratory conditions, acclimatizing for one week at a room temperature of 23-25°C with a 12 hour dark/light cycle. They were allowed to feed and drink ad *libitum* on standard pellet diet and tap water.
Nifedipine (Sigma Aldrich product no. N7634) and Dimethyl sulfoxide (Sigma Aldrich product no. D5879) was purchased from the local Distributors. Aqueous fruit extract of TT was made from fruits of TT after plant identification from herbarium of Quaid-e-Azam University. Fruit about 500 gms was powdered and soaked in 1 liter of distilled water overnight. After filtration the filtrate was dried at reduced temperature using rotary evaporator and then incubated at room temperature for 2 days. The dry mass served as an aqueous fruit extract of TT.[19]
Gavage needle was used to administer the substances orally. Group A (Control) was administered 0.5 ml distilled water and 1ml of Dimethyl sulfoxide (DMSO) per rat, once daily, orally, for 8 weeks. Group B (Nifedipine only group) was given Nifedipine at a therapeutic dose of 50 mg/kg body weight, dissolved in 1ml of DMSO, once daily, orally for 8 weeks. Group C (TT only group) was given TT aqueous extract 6mg/kg/rat, once daily, orally, for 8 weeks. Group D (Nifedipine plus TT) was given Nifedipine and TT together in the same doses, orally, once daily as were given to Group B and C

respectively for 8 weeks.

At the end of 8 weeks animals in all groups were anesthetized and sacrificed. Peritoneal cavity was opened by lower abdominal transverse incision; testes were removed, washed with saline, blotted and weighed on digital balance.[19,20]They were fixed in 10 % formalin. Transverse sections from the middle of testis were taken. Slides were prepared after cutting 5 µm thick serial sections on rotary microtome and staining with Haematoxylin and Eosin (H&E). Transversely cut seminiferous tubules were selected in the slides to measure the diameter of seminiferous tubules under light microscope at the power of 10 and height of germinal epithelium at the power of 40 with ocular micrometer. In each tubule both maximum and minimum diameters were measured and their mean was taken. Height of germinal epithelium was measured from the basement membrane to surface of the epithelium in the same tubules. Epithelial Height was measured at 4 places, equidistant from each other, in each cross-section and their mean was calculated.[21]

Mean and standard deviation (SD) was calculated for height of germinal epithelium and diameter of seminiferous tubules. The data was entered and analyzed using SPSS 22. The statistical significance of difference across the groups was determined by applying One way analysis of variance (ANOVA) followed by Tukey HSD (honestly significant difference) post hoc test to compare the mean differences among various pairs of groups. A *p*–value of <0.05 was considered as statistically significant.

Results

On macroscopic examination the testes were normal looking in all four groups. Microscopic examination of control group exhibited densely packed seminiferous tubules with little stroma in between, lodging Leydig cells. Control group, TT only group and Nifedipine plus TT group revealed evidence of active seminiferous tubules undergoing spermatogenesis and spermiogenesis(figure1,2 and 4).Different stages of spermatogenesis with spermatogonia, primary spermatocytes, round spermatids, elongated spermatids, and spermatozoa were visible in all sections of above mentioned groups. Spermiogenesis was shown by the presence of residual bodies and tails within lumen of seminiferous tubules.

In Nifedipine only group most of the tubules exhibited reduced tubular diameter and height of the germinal epithelium with depletion of few cellular layers within seminiferous epithelium. (fig3)Spermatogenesis was seen to be mostly arrested at elongating spermatid stage while Leydig cells appeared to be normal. There was no evidence of testicular degenerative changes.

Table I. Comparison of Parameters Among Four Groups By One Way ANOVA

Variables	Control group	Nifedipine only Group	TT only group	Nifedipine +TT group	*p*-value
Height of seminiferous epithelium (µm)	109.06 ± 10.54	102.36 ± 3.27	134.48 ± 13.55	129.49 ± 4.81	<0.001*
Diameter of seminiferous tubules (µm)	428.37 ± 59.15	389.79 ± 40.72	575.25 ± 39.11	547.51 ± 48.00	<0.001*

Where all values are expressed as mean ± standard deviation.

* Statistically significant values.

Table II. Group Comparison of Mean Differences in Diameter of Seminiferous Tubulesand Height of Germinal Epithelium By Post Hoc Tukey Test

Group Comparison	Diameter of seminiferous tubules (µm)		Height of germinal epithelium (µm)	
	Mean Difference	*p*-value	Mean Difference	*p*-value
Group B vs Group A (Nifedipine vs. control group)	38.58	0.011*	6.70	0.025*
Group C vs Group A (TT vs. control group)	146.88	<0.001*	25.41	<0.001*
Group D vs Group A (Nifedipine plus TT vs. control group)	119.14	<0.001*	20.43	<0.001*
Group D vs Group B (Nifedipine plus TT vs. Nifedipine group)	157.72	<0.001*	27.13	<0.001*
Group D vs Group C (Nifedipine plus TT vs. TT group)	27.74	0.112	4.98	0.150

*Statistically significant values

Discussion

Calcium Channel Blockers, most commonly used antihypertensive[22] are long been known for induction of male infertility.[23] They have been reported to induce temporary infertility either by inhibition of Leydig cell steriodogenesis, depression of spermatogenesis, destruction of the germinal cell, inhibition of sperm function or its metabolism.[24]

Our study revealed that control group, TT only group and Nifedipine plus TT group revealed evidence of active spermatogenesis but in Nifedipine only group most of the tubules exhibited reduced tubular

diameter and height of the germinal epithelium.

The main finding in Nifedipine only group showing reduction in spermatogenesis was supported by parallel studies of Latif *et al* , who had shown significant decrease in the height of seminiferous epithelium and diameter of tubules in adult male rats after giving them amplodipine for 50 days.[25]But these effects are reversible after discontinuing the drug. Similar study was reported by Adebayo A *et al* who depicted in his study the dose dependent delirious effect of amlodipine on seminiferous epithelium adult Wistar rats.[26] Lee *et al*[21] conducted study on prepubertal male mice which were given Nifedipine and Ethosuximide for 20 days. It caused a significant fall in sperm production and spermatogenic arrest mainly at the elongating spermatid stage in a dose-dependent fashion. Both T- and L-type Ca^{2+} channel blockers interfere with normal spermatogenesis and steroidogenesis firstly by blocking post meiotic germ cell maturation and/or by abolishing StAR protein expression, contributing to male infertility.[23] Sperm structure influences its motility and is also a predictor of overall health of the testes.[13] Our findings of Nifedipine only group coincided with above mentioned researches .Reduction in spermatogenesis with the use of Nifedipine may be either secondary to decrease in serum testosterone or in pituitary hormones or direct harmful effects of the Nifedipine on germ cells.[25]

There was significant increase in the height of seminiferous epithelium and diameter of tubules in TT only group because of the positive effect of aqueous extract of TT on germinal epithelium.TT has been shown to increase the height of seminiferous epithelium and diameter of seminiferous tubules as it increases the level of testosterone and LH in the body which promotes spermatogenesis.[27]Jashni *et al* reported in his study that estradiol glycosides in TT extract(PTN) cause increase in the testosterone levels by enhancing the production of androgens.[18] Histological observations made by Esfandiari *et al* after giving high dose of TT to mature and immature rats revealed increased height of germinal epithelium and diameter of seminiferous tubules in both experimental groups as compared to control groups.[28] Research conducted on rabbits by Al-Rubii et al revealed similar results.[29] Jashni *et al*[18] showed that the mean number of primary spermatocytes increased with TT dose of 10 mg/kg. In our study the histological changes became evident at 6mg/kg body weight given for same duration as above. This difference in the response might be due to geographical difference in the origin of TT plants[30]or due to aqueous extract of TT unlike alcoholic extract as used by Jashni *et al* in his study. The group Nifedipine plus TT has significant increase in the height of germinal epithelium and diameter of tubules with respect to Nifedipine only group. Nifedipine couldn't suppress spermatogenesis completely. The extract of TT is reported to increase serum testosterone. One of the suggested mechanisms is that the level of testosterone is augmented by increasing LH and the GnRH.[17]It can be direct conversion of PTN to DHEA in the body which then undergo further conversion to testosterone.[31]

TribulusTerrestris protective effects should be seen at different doses in male gonads along with changes in serum hormonal level. Cellular mechanism of action of Tribulus should be explored.

Conclusion

The current study has led us to presume that herb *Tribulus Terrestris* has protective effect on Nifedipine induced damage on microstructure of testes. Therefore temporary infertility in males induced by commonly used calcium channel blockers, Nifedipine, intake can be prevented by co-administration of *Tribulus terrestris* aqueous extract as has been confirmed by histological parameters.

Limitation of our study is non estimation of serum Testosterone and LH, which could have augmented the histological changes seen in testis

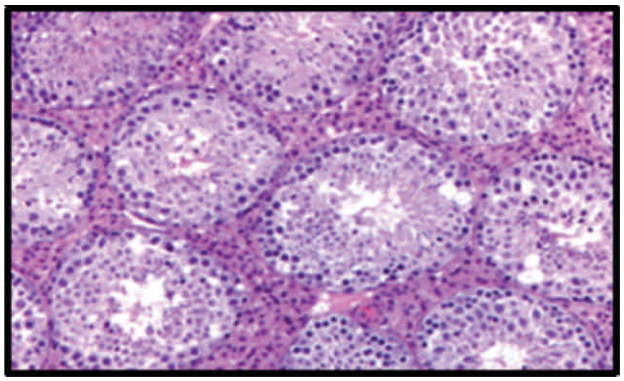

Fig 1: Photomicrograph of Testicular Tissue Showing Normal Histology in Control Group (H & E Stain x 300 approx.)

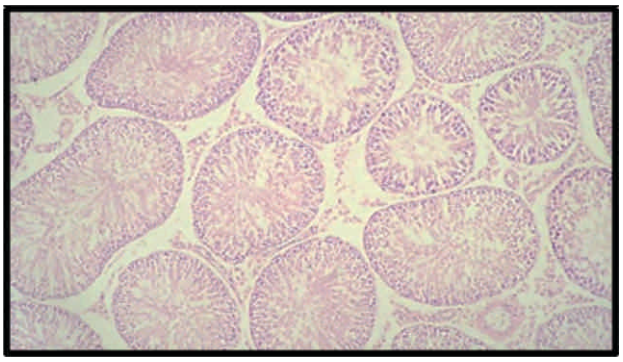

Fig 2 : Photomicrograph of Testicular Tissue Showing Testicular Histology In *Tribulusterrestris* Treated Group (H & E Stain X 300 Approx.)

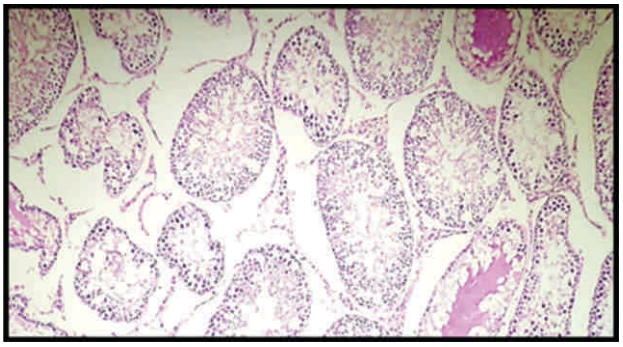

Fig 3: Photomicrograph of Testicular Tissue Showing Histological Changes In Nifedipine Treated Group (H & E Stain X 300 Approx.)

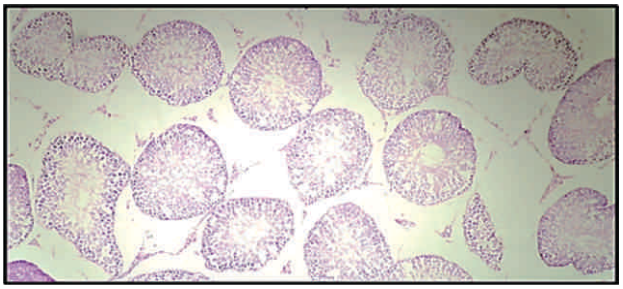

Fig 4: Photomicrograph of Testicular Tissue Showing Testicular Histology In *Tribulus Terrestris* Plus Nifedipine Treated Group (H & E Stain X 300 Approx.)

microstructure. In addition technical limitations prevented us to study the cellular mechanism of *Tribulus Terrestris* at Leydig cell level.

Recommendations

Tribulus Terrestris protective effects should be seen at different doses in female gonads along with changes in serum hormonal level.

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