ORIGINAL ARTICLE

PROTECTIVE EFFECT OF *TRIBULUS TERRESTRIS* ON NIFEDIPINE INDUCED CHANGES IN MALE SEX HORMONES

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Background: Therapeutic administration of calcium channel blockers (CCBs) has been correlated with iatrogenic male infertility. Any agent that may increase testosterone production and spermatogenesis can be helpful for enhancing fertility in patients taking CCBs. The objective of this study was to determine the protective effect of Tribulus on nifedipine induced changes in serum luteinizing hormone (LH) and testosterone in male rats. **Methods:** This experimental study was conducted at Shifa College of Medicine, Islamabad in 2014. The study was done on 120 adult male Sprague Dawley rats, divided into 4 groups. Group A received 0.5 ml distilled water once daily orally, group B was administered Nifedipine 50 mg/Kg/rat orally, group C was administered Tribulus aqueous extract 6 mg/Kg/rat once daily, and group D was given both Nifedipine and Tribulus. All doses were given for 8 weeks. After 8 weeks, serum testosterone and serum LH were measured in all groups. **Results:** The mean serum LH, mean serum testosterone, and *p*-values for Nifedipine group as compared to the control group were 0.928 and <0.001 respectively; for Tribulus group in regard with the control group were <0.00 and <0.00); for the Nifedipine+Tribulus group were (0.00) and (<0.00) respectively as compared to control group. Nifedipine+Tribulus group had $p<0.00$ for mean serum LH and $p<0.00$ for mean serum testosterone, with respect to Nifedipine. **Conclusion:** Tribulus can be used as a protective drug for the calcium channel blocker induced delirious changes in sex hormones (LH and testosterone) in male rats.

Keywords: Aphrodisiacs, Infertility, Calcium channel blockers, Nifedipine, Tribulus Terrestis

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INTRODUCTION

Calcium channel blockers (CCBs) are among the most effective drugs used for the control of blood pressure in hypertensive patients of all age groups. The CCBs are classified into three subgroups on the basis of chemical structure; benzothiazepines, phenylalkylamine and dihydropyridines (DHPs).[1] Nifedipine is an important member of DHPs.[2] It acts by blocking influx of calcium ions through the L-type voltage gated calcium channels (VGCCs) of smooth muscle cells (SMC) of blood vessels. It reduces the blood pressure by decreasing peripheral vascular resistance mainly at the level of small arterioles.[3,4]

Similarly calcium is required for the normal spermatogenesis and for normal sperm motility and fertilization. Therefore, therapeutic administration of CCBs has been correlated with iatrogenic male infertility. This infertility may be due to decreased testosterone production, suppression of spermatogenesis or decrease in sperm motility. Any agent that may increase testosterone production and spermatogenesis can be helpful for enhancing fertility in patients taking CCBs.[5,6]

Different factors with stimulatory or inhibitory effects on testosterone production have been studied in animal models but *Tribulus terrestris* (TT) and Yohimbine have been reported to have proven pharmacological effect. The protective effect of Tribulus still needs to be discovered.[7]

This study was planned to determine the protective effects of TT on nifedipine-induced changes in serum luteinizing hormones and serum testosterone in rats.

MATERIAL AND METHODS

The study was a randomized control trial conducted in 2014, in the Research Laboratory of Shifa College of Medicine, in collaboration with National Institute of Health (NIH), Islamabad.

One hundred and twenty adult male Sprague Dawley rats that weighed about 200±50 g each were maintained under standard laboratory conditions at 23±2 °C with constant light-dark cycle and were provided with standard rat diet and water *ad libitum*. Rats were randomly divided into four equal groups. Group A (Control), Group B (Nifedipine), Group C (TT) and Group D (Nifedipine plus TT).

Control group was administered placebo that was 0.5 ml distilled water and 1 ml of Dimethyl sulfoxide (DMSO)/rat once daily orally for 8 weeks, using gavage needle. Nifedipine group was given Nifedipine 50 mg/Kg/rat once daily, orally dissolved in 1 ml of DMSO for 8 weeks, using gavage needle for a period of eight weeks.[8] TT group was given TT aqueous extract 6 mg/Kg/rat once daily orally, with the help of gavage needle, for 8 weeks.[9] Nifedipine (in the same dose as group B) and TT (in the same dose as group C) were given together to rats for 8 weeks. The drug was given once daily.

After eight weeks, terminal intracardiac sampling was done for the assay of serum parameters. Serum was separated by centrifugation, frozen and stored at -80 °C until assayed. Serum testosterone (ng/ml) and serum luteinizing hormones (IU/L) were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits.

The data were processed statistically using SPSS-21. The arithmetic mean and standard deviation of serum LH and testosterone were calculated. Difference in mean among control and treated groups was calculated by ANOVA followed by post hoc Tukey's test.

RESULTS

Serum testosterone and serum LH levels in all 4 groups after 8 weeks of study are presented as Table-1. There was significant decrease in the serum testosterone levels in nifedipine group as compared to control group, with corresponding rise in LH which is not statistically significant with respect to control group.

Table-2 illustrates the mean differences in serum testosterone and serum LH levels when Tukey's HSD post hoc analysis was applied.

Table-1: Comparison of Mean±SD of serum LH and serum Testosterone

Variable	Control Group	Nifedipine group	Tribulus Terrestris group	Nifedipine+TT group	*p*
Serum LH (IU/L)	1.26±0.54	1.18±0.42	1.75± 0.65	1.73±0.42	<0.001*
Serum Testosterone (ng/ml)	3.26±0.38	2.57±0.31	4.38±0.38	4.17±0.40	<0.001*

*Significant

Table-2: Group comparison of mean differences in serum LH and serum testosterone levels by post hoc Tukey's test

	Serum LH (IU/l)		Serum Testosterone (ng/ml)	
Group Comparison	**Mean Difference**	***p***	**Mean Difference**	***p***
Nifedipine *vs* control group	0.0823	0.928	0.6946	<0.001*
Tribulus Terrestris *vs* control group	0.4846	0.003*	1.122	<0.001*
Nifedipine plus Tribulus Terrestris *vs* control group	0.4640	0.004*	0.9050	<0.001*
Nifedipine plus Tribulus Terrestris *vs* Nifedipine group	0.5463	<0.001*	1.599	<0.001*
Nifedipine plus Tribulus Terrestris *vs* Tribulus Terrestris group	0.0206	0.999	0.2170	0.117

*Significant

DISCUSSION

The results of our study are in parallel with the study conducted on adult male rats by Latif *et al*, which illustrated that in the treated group there was significant reduction in serum testosterone levels by amlodipine.[10]

CCBs block steroid production because of its imperative nature in diverse cellular processes as increase in intracellular Ca^{2+} through VGCCs induced by LH is obligatory for testosterone production. Ca^{2+} ions induce the transcription of steroidogenic acute regulatory (StAR) protein necessary for normal steroidogenesis.[11] Three moles of NADPH are required per mole of cholesterol converted to pregnenolone by P450scc. The enzyme complex of hydroxysteroid dehydrogenases require NADH or NADPH for their proper functioning.[12] Ca^{2+} ions are transported in the mitochondria for enhancing the synthesis of NADH and NADPH in order to modify the redox state of mitochondria.[13,14]

The role of Ca^{2+} in germ cell transcription is ascertained by the fact that human chorionic gonadotropin (hCG) induced StAR protein expression and steroid production are augmented by Ca^{2+} ions and the increase in StAR protein and steroid hormone are diminished leading to reversible male sterility by blocking L-type Ca^{2+} channels using verapamil or nifedipine or that inhibition of the Ca^{2+}/calmodulin protein kinase pathway greatly worsen StAR expression in Leydig and adrenal cells.[11,15]

In the view of above discussion, the instituted role of Ca^{2+} for normal steroidogenesis, sperm motility, spermatogenesis, chemotaxis, acrosomal reaction, capacitation and process of fertilization cannot be refuted.[16,17] In our study nifedipine reduced the level of testosterone in the treated group without effecting LH.

Lee *et al* conducted study using nifedipine and ethosuximide on prepubertal rats showing significant fall in serum testosterone production illustrating impaired steroidogenesis.[18]

We conducted *in vivo* study, but Lee *et al* in their *in vitro* work on Leydig cells concluded that mibefradil has inhibitory actions on steroidogenesis in mouse Leydig cells with the involvement of Ca^{2+} entry via the T-type Ca^{2+} channel in the plasma membrane of Leydig cells. They also explained that effects of CCBs on hCG- or cAMP-stimulated steroidogenesis are facilitated by transcriptional suppression of the StAR gene in mouse Leydig tumour cells. En masse, these results clearly showed that Ca^{2+} entry is needed for steroidogenesis in Leydig cells.[19] These findings are in parallel with our results.

Albers *et al* conducted study on the chronic effect of nifedipine on eleven Caucasian males for an average of three years in comparison to 11 control males

which matched for each other for confounding factors like age, height, weight, activity level, cardiovascular status, and calcium intake. No significant differences between groups were noted in serum testosterone, parathyroid, vitamin D and calcitonin levels.[20]

Similar to the above study Iranloye *et al* investigated the effect of nifedipine on serum testosterone by taking twenty-four male rats. Drug was given orally for 30 days and serum testosterone level were assessed which remained unaltered in treated rats.[6] These differences in response might be due to differences in the experimental models, route of drug administration and duration of drug administration in various studies.

Nifedipine plus TT has significant increase in the levels of testosterone and LH compared to the control group and Nifedipine group, but the values are not significant compared to TT group. These results showed that TT can be used as a preventive or protective drug for the CCB-induced infertility in male hypertensive patients.

CONCLUSION

Tribulus can be used as a protective drug for the calcium channel blocker induced infertility in male hypertensive patients.

LIMITATIONS OF THE STUDY

1. Serum FSH was not measured due to financial constraints.
2. *In vitro* study to elucidate the cellular mechanism of *Tribulus terrestris* at Leydig cell level was not done due to technical limitations and financial constraints.

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