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NIFEDIPINE ON;

EFFECT OF NIFEDIPINE ON SERUM LUTEINIZING HORMONE AND SERUM TESTOSTERONE IN MALE SPRAGUE DAWLEY RATS.

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ABSTRACT… Background: The most potent and effective drugs used for the management of blood pressure in hypertensive patients are Calcium channel blockers (CCBs). Nifedipine, a CCB, acts by blocking entry of calcium ions all the way through the voltage gated calcium channels (VGCCs) of L-type present in the smooth muscle cells of blood vessels and reducing the blood pressure by decreasing the peripheral vascular resistance. **Objectives:** The study objective was to determine the effect of nifedipine on serum luteinizing hormone (LH) and serum testosterone in male Sprague Dawley rats. **Study Design:** Animal experimental study. **Setting:** All experiments were conducted at the Research laboratory of Shifa College of Medicine, Islamabad along with National Institute of Health (NIH), Islamabad. **Period:** October, 2012 to April, 2014. **Methods:** The study was done on adult male Sprague-Dawley rats (N= 60) aged 90-120 days old and their body weights varied between 200 + 50 grams. Rats were divided into two groups (n=30). Group A was administered 0.5 ml distilled water/rat daily orally, group B was administered orally with nifedipine 50 mg/kg/rat dissolved in 1ml of DMSO. All the doses were given to rats for 8 weeks. After 8 weeks, serum luteinizing hormone and serum testosterone were measured in both groups. **Results:** In Nifedipine treated group, serum testosterone was significantly decreased and serum LH was unaffected as compared to the control group. **Conclusion:** Nifedipine has adverse effects on male fertility as it decreases serum testosterone level.

Key words: Serum Testosterone, Serum LH, Nifedipine.

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INTRODUCTION

CCBs are classified into 3 subgroups based on the chemical structure. Though all CCBs share the same mechanism of action of blocking L-type VGCCs, dihydropyridines alter in important pharmacological properties like cardio-depressant activity and tissue selectivity which makes them one of the most prescribed drugs of the world.[1] These drugs became the best-selling antihypertensive drugs during the late 1980s and early 1990s.[2]

The 1,4-dihydropyridine nifedipine is a vasoselective, lipophilic dihydropyridine type of CCBs.[3] Nifedipine acts by blocking calcium (Ca^{2+}) influx through voltage-dependent L channels without affecting calcium release from the sarcoplasmic reticulum.[5] Diminish in the intracellular calcium concentration reduces smooth muscle cells tone in the blood vessels leading to decline in peripheral vascular resistance. This decline in peripheral resistance at the level of small arterioles causes decrease in systemic blood pressure. The maximum daily dose of nifedipine to relieve hypertension is about 120 mg in humans.[6,7]

Since introduction of nifedipine into the clinical medicine, it has undergone a number of modifications over time to upgrade the pharmacokinetic profile and administration regimen from three times daily to once daily.[8]

Anti-hypertensive drugs have been made known to adversely affect male fertility. Treatment of CCBs to relieve HTN causes reversible male infertility as illustrated by the use of nifedipine, which resulted in reversible deleterious effect on sperm

functions in male rats.[9,10] Analogues of nifedipine cause reversible infertility. These analogues work in a different way with respect to their antifertility pursuit which is achieved by varying the cell metabolism thereby directly affecting the motility of sperm which is accountable for male fertility.[11] Ninety days subsequent the discontinuation of these medications, absolute recovery of sperm function occurs in patients on CCBs.[12] Infertility of males has numerous basis, it has been advocated that CCBs contribute to male infertility, proposing the idea that testis calcium homeostasis in is intimately connected with male reproductive ability.[9,13] Therefore therapeutic intervention of CCBs to be done with vigilance due to its probable undesirable side effects on male infertility.[14]

Fertility in males is dependent upon ample production, proper storage and transportation of sperms. Therefore disruption of this fertility may occur via the inhibition of steroidogenesis in Leydig cell, depressed spermatogenesis, germinal cell destruction, sperm function inhibition and collapse of transport of normal sperms.[15,16] Physiological significance of VDCCs in the process of spermatogenesis and steroidogenesis is stated by the facts that calcium is required for germ cell development. Stimulatory actions of luteinizing hormone and follicle-stimulating hormone (FSH) on development of testis and steroidogenesis involve intracellular Ca^{2+}regulated via VDCCs present in the Leydig and Sertoli cells. Any improper functioning of calcium channels in the above mentioned cells may lead to a substantial loss of spermatogenesis and steroidogenesis.[17]

Reduction in plasma FSH and testosterone occur with amlodipine treatment. No effects on serum LH are reported with amlodipine therapy.[18] Similarly with nifedipine therapy reduction of serum testosterone with no effect on serum LH has been reported in studies conducted on rats.[14]

Reduction in testosterone levels with the use of nifedipine shows either a direct action of CCBs on testes influencing biosynthesis of testosterone at Leydig cell level or an indirect mechanism influencing the hormonal milieu at the hypothalamic-pituitary axis. In the same manner, decline in spermatogenesis may be either resulting to decrease in serum testosterone, pituitary hormones or direct injurious actions of the nifedipine on germ cells.

MATERIAL AND METHODS

The study was conducted from October, 2012 to April, 2014, at the Research Laboratory of Shifa College of Medicine, Islamabad in collaboration with National Institute of Health (NIH), Islamabad.

Adult male Sprague Dawley rats (60) weighed about 200±50 g each, taken from National Institute of Health, Islamabad, were maintained under temperature at 23±2 °C with constant light dark cycle and were supplied with standard rat diet and water ad libitum. Group A (control) was given 0.5 ml distilled water/rat through oral route and group B (experimental) was given Nifedipine 50 mg/kg once daily, orally dissolved in 1ml of DMSO for 8 weeks, using gavage needle.

Animals were sacrificed one day after the last dose administration according to protocols and ethical procedures. Blood samples were collected under deep ether anesthesia through intracardiac sampling.

Serum Testosterone, Enzyme immunoassay was done using Cayman's chemicals EIA Testosterone kit, Catalogue Number: 582701. Enzyme immunoassay of serum LH was done by Luteinizing Hormone EIA kit, Catalogue Number: CSB-E1265. SPSS version 21 was used for processing data statistically. The arithmetic mean and standard deviation of all observations were calculated. Difference in mean among control and treated groups was calculated by 'independent t-test'.

RESULTS

At the end of 8 weeks of study arithmetic mean and standard deviation of serum testosterone and serum LH in both control and Nifedipine treated groups are summarized in Table-I.

The p-values for mean serum LH and mean serum testosterone for Nifedipine treated group with reference to the control group were 0.928 and

<0.001 respectively, indicating that in this group serum testosterone has decreased significantly with no statically significant change in mean value of serum LH in both the groups.

Mean ± SD values of serum Luteinizing hormone and serum Testosterone			
Variable	**Control Group (n = 30)**	**Nifedipine Group (n = 30)**	**p Value**
Serum Luteinizing hormone IU/l	1.26+0.54	1.18+.42	0.928
Serum Testosterone ng/ml	3.26+.38	2.57+.31	< 0.001

Table-I. Mean of serum luteinizing hormone and serum in control and nifedipine treated groups

DISCUSSION

VGCCs are found in various tissues in the body like male reproductive tissue. Increase in intracellular calcium through voltage gated calcium channels of Leydig cells induced by LH is essential for testosterone production by the testes. The process involves attachment of LH to hormone receptors on Leydig cells initiating the activation of adenylate cyclase, resulting in an attendant increase in cyclic adenosine mono phosphate (cAMP), ensue by the entry of Ca^{2+}ions via VGCCs found in the cell membrane of Leydig cells and increase in protein kinase A (PKA) catalyzed by cAMP.[19] PKA increases the rate of hydrolysis of cholesteryl esters making more cholesterol available for testosterone production by Leydig cells. Ca^{2+} ions also induce transcription of Steroidogenic acute regulatory protein (StAR) needed for steroidogenesis. SimilarlyCa^{2+}, is required for the normal spermatogenesis and for 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.[20]

In our study we measured serum testosterone and serum LH in Nifedipine treated and control groups by ELISA. The results showed that there was significant decrease in the serum testosterone levels in nifedipine group as compared to the control group with correspondingly raise in LH which is not statistically significant with respect to control group.

These result are in parallel with the study conducted on adults male rats by Latif et al who illustrated that in the treated group there was significant diminution in serum testosterone by amlodipine, indicating either a straight injurious consequence of CCBs at level of Leydig cells or an not direct effect by disrupting the hormone production at hypothalamo-pituitary axis because the GnRH mediated LH discharge from pituitary gonadotrophs is calcium reliant. But LH levels are not significantly effected in the study ruling out this factor of fall in serum testosterone in our study.[21]

So as in our study nifedipine reduced the level of testosterone in the treated group without effecting LH. In favor of our study no noteworthy outcome of nifedipine or nicardipine on LH has been reported by Feely et al.[22]

To observe the in vitro effect of intracellular calcium on steroidogenesis in Leydig cell, Latif R et al designed his study to see these actions through amlodipine which is a calcium channel blocker. Leydig cells were isolated, purified followed by incubation for 3 hours with and without the drug. Estimation of cytosolic calcium was carried out. The results illustrate significantly reduced steroidogenesis ($P<0.05$) and low intracellular calcium in rats exposed to amlodipine.[23]

Lee et al conducted study using nifedipine and ethosuximide on prepubertal rats. Drugs were administered for 20 days. Morphological changes in the testis were recorded. The cell counts were done on sperm and Leydig cells. Serum testosterone level was carried out by radioimmunoassay (RIA) and reverse transcription and polymerase chain reaction (RT-PCR) were used to estimate StAR protein mRNA. Results showed significant fall in serum testosterone production illustrating impaired steroidogenesis. The number of Leydig cells and the amount of StAR protein mRNA were less as compared to the controls. He depicted clear dose

dependent relationship of CCBs and testosterone concentration but LH concentrations were unaffected at all doses.[14] VGCCs may be linked with LH induced steroidogenesis in Leydig cells as in other steroid producing cells.[24]

We conducted In vivo study but Lee et al in his In vitro work on Leydig cells concluded that mibefradil has inhibitory actions on steroidogenesis in Leydig cells with the participation Ca^{2+} ions through entry via the T-type channel in the outer membrane of these steroid producing cells. He explained that effects of CCBs on hCG- or cAMP-stimulated steroidogenesis are augmented by transcriptional suppression of the StAR gene in mouse Leydig tumor cells. En masse, these results depicted that Ca^{2+} entry is required for steroidogenesis in Leydig cells.[25] These findings are in parallel with our results.

Conflicting with our results Struthers AD et al conducted study on the effect of nifedipine on nine normotensive male subjects. In a randomized single blind study, nifedipine was administered or the matching placebo was given. Nifedipine had no significant effect on serum LH and testosterone. The study thus concluded that nifedipine does not suppress the production of LH and testosterone and does not affect male fertility.[26]

Similar to the above study Iranloye et al explored the effect of the nifedipine on twenty four male rats which were randomly divided into three groups. Control group were given distilled water; Experimental group received nifedipine 0.57 mg/kg; and Recovery group received 0.57 mg/kg. Doses were administered for one month. Animals in Recovery group were allowed another month after drug withdrawal for purpose of recovery. Sperm count, motility, morphology and serum testosterone level were evaluated. Results showed that nifedipine administration significant decreased the testicular weight and epididymis ($p < 0.05$). Sperm count and motility were also significantly decreased ($p < 0.05$). However serum testosterone was unaffected in nifedipine treated rats. The study concluded that nifedipine may have reversible damaging effects on sperm motility in rats which are not by suppressing testosterone production by Leydig cells.[10]

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 and last but not the least the choice of CCB used in experiment.

CONCLUSION

Nifedipine, a calcium channel blocker, may have negative influence on male fertility owing to its effect on testosterone secretion by Leydig cells of testis.

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We are not makers of **history**.
We are made by **history**.

"Martin Luther King, Jr."

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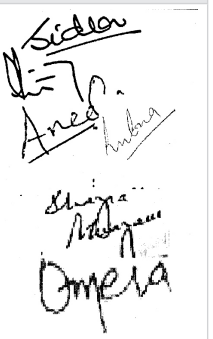
1	Sidra Hamid	Literature search, Study design, Experimental work.	
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