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Original Research

Dose-dependent reproductive toxicity of nimodipine in male rats

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Male infertility; Nimodipine; Rats; Spermatogenesis

Abstract

Objective: Nimodipine is a calcium channel blocker that inhibits calcium ion transfer into cells. It has greater effects on cerebral arteries due to high lipophilicity and used for cerebral hemorrhage. In this study it was aimed to investigate the dose-dependent effect of nimodipine on the testicular tissue of male rats.

Methods: Twenty four male rats were allocated into four groups (6 rats in each); first group served as control, the others received 20, 40, 80 mg/kg/day of nimodipine, respectively, for 30 days. At day 31, the animals were sacrificed, the testicular tissues were removed, and sperm was collected from epididymis and prepared for analysis.

Results: Significant and dose-dependent decrease in sperm count and motility associated with morphological changes were reported in addition to progressive damage in the epididymis and testicular tissue architecture of treated animals compared to controls.

Conclusion: Nimodipine decreases sperms count and activity in a dose-dependent pattern, associated with disarrangement testicular structural elements of male rats; this confirms the class effect of calcium channel blockers in this respect.

INTRODUCTION

An estimated 40 to 90% of male infertility is due to deficient sperm production of indefinable origin [1]. Infertility may also be due to abnormal sperm morphology and insufficient sperm motility [2]. Different factors that are responsible for male infertility, including calcium ions homeostasis, are implicated in diverse cellular functions in both germ cells and testicular tissues, particularly mediating the responses to endocrine hormones and local regulators in genital tracts [3, 4]. Many regulatory functions in the spermatozoa, including acrosome reaction [5], capacitation [6], motility [7, 8], and hyperactivity are regulated by cytoplasmic calcium [9, 10].

Previous reports showed that amlodipine decreases sperm count in rats [11]. In humans, long term treatment with the calcium channel blockers (CCBs), such as amlodipine and nifedipine, resulted in azoospermia in the semen and few non-motile sperms in testicular sperm extraction [12]. This indicates that long term use of these CCBs not only inhibits spermatogenesis but it also impairs sperm motility [13].

Problems with sexual function have been a long-standing concern in the treatment of hypertension and other cardiovascular diseases, and may influence the choice of treatment regimens and decisions to discontinue drugs [14]. However, despite their substantial cardiovascular selectivity, reports exist for infertility in males who are using CCBs [13, 15]. The effect of nimodipine in causing infertility is not clarified yet and needs to be elucidated; accordingly, the present study was designed to evaluate the dose-response relationship of the expected nimodipine reproductive toxicity in male rats.

MATERIALS AND METHODS

Twenty four male rats (180-250 g body weight) are brought from the animal house of College of Pharmacy, University of Baghdad, and used in the present study.

They are housed in the animal house of the College of Pharmacy, University of Basra, in standard conditions of temperature (23-25°C), humidity and 12:12 hr light-dark cycle with free access to standard laboratory chow and tap water. The rats were randomly allocated into 4 groups (6 rats in each) and treated as follow: the first three groups received daily oral doses (20, 40 and 80 mg/kg) of nimodipine (Bayer, Germany) aqueous solution, using oral gavage needle, for 30 days; the fourth group received vehicle and saved as control. The research protocol was approved by the animal research ethics committee of the College of Pharmacy, University of Baghdad, in accordance with the internationally accepted legislations in this respect.

Sperm analysis

Sperm samples were obtained from the cauda epididymis and analyzed using conventional method [16]; after removal of testicular tissue, the adherent fat, blood vessels and connective tissue were cut away and the organ from each animal was placed in a hollow plate. The sperms were released by cutting the cauda epididymidis longitudinally with a pair of fine-pointed scissors and compressing with forceps, and were therefore deposited free of epididymal tissue into the cavity. Epididymal sperms were obtained by mincing the epididymis with anatomical scissors in 5 ml of physiological saline. Briefly, sperm motility was assessed by placing 10 µl of sperm suspension on slide for microscopic evaluation, using Neubauer hemocytometer chamber, at a magnification of x40. About 100 sperm cells were examined from five different fields in each sample and classified as either motile or immotile and expressed as percentage.

Histological processing

The testes, epididymis and seminal vesicle from all rats were taken immediately after laparatomy of the animals. Histological processing was done as described by Akpantah *et al* [17]. The organs were cut in slabs of about 0.5 cm thick transversely and fixed in Bouin's fluid for a day, after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Serial sections were cut using rotary microtome at 5 µ. Slides were prepared from these tissues. The slides were de-waxed and passed through absolute alcohol (2 changes); 70% alcohol and then to water for 5 min. The slides were then stained with hematoxylin and eosin (H&E), and then examined under light microscope.

Statistical analysis

The results were expressed as mean ± SEM. Statistical comparisons were done using unpaired t-test and ANOVA for comparisons of data in the control group and the experimental groups. Differences with $P < 0.05$ were considered significant.

RESULTS

Effects of nimodipine on sperm counts, activity and quality

Treatment of rats with different doses (20, 40 and 80 mg/kg) of nimodipine significantly decreased both sperms count (12.4%, 16.5% and 22.9%, respectively) and activity (14.5%, 25.9% and 37.8%, respectively) compared to control group (Table 1). Comparison between the effects of the increase in doses revealed significant differences between the effects of nimodipine doses in this respect.

The percent of normal sperms was significantly decreased in the groups treated with 40 and 80 mg/kg nimodipine compared with controls (Table 2). Moreover, the rats treated with 40 and 80 mg/kg nimodipine expressed significant increase in percentage of abnormal spermatozoa, which include headless or tailless sperms (Figs.1-3), especially at the group received nimodipine dose of 80 mg/kg.

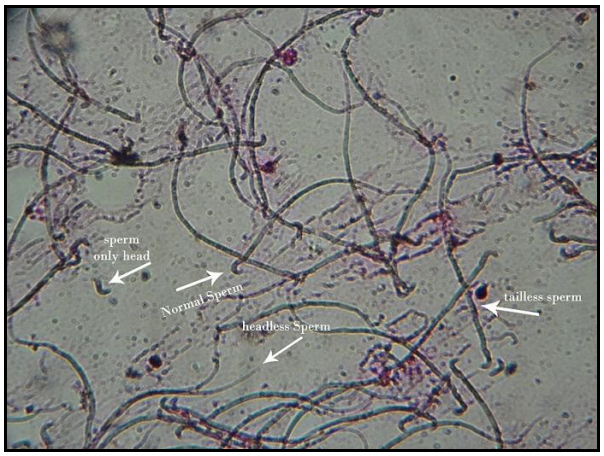

Figure 1. Effect of 20 mg/kg nimodipine on sperm morphology

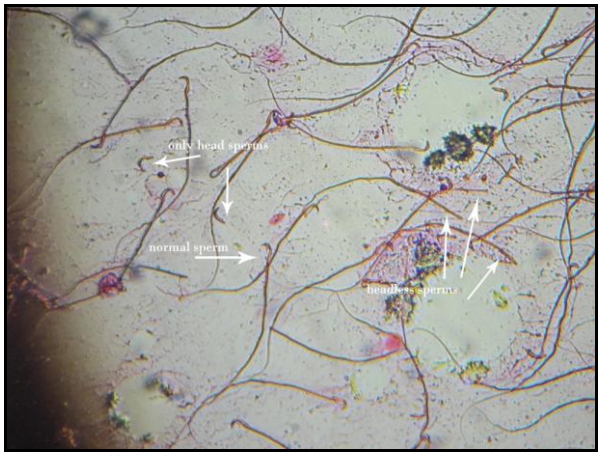

Figure 2. Effect of 40 mg/kg nimodipine on sperm morphology

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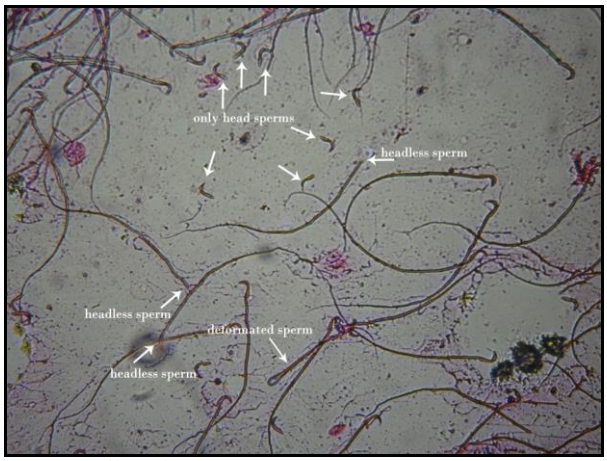

Figure 3. Effect of 80 mg/kg nimodipine on sperm morphology

Table 1. Dose-dependent effects of nimodipine on sperm count and sperm activity in male rats

Treatment groups (n = 6)	Sperm count (10^6)	Sperm activity (%)
Control	2.41 ± 0.21	74.33 ± 3.14
Nimodipine 20 mg/kg	2.12 ± 0.16*[a]	63.59 ± 5.95*[a]
Nimodipine 40 mg/kg	2.02 ± 0.21*[b]	55.10 ± 5.9*[b]
Nimodipine 80 mg/kg	1.86 ± 0.18*[c]	35.51 ± 8.96*[c]

Values represent mean ± SEM; n: number of animals in each group; *significantly different compared to control ($P < 0.05$); values with non-identical superscripts (a, b, c) within the same parameter are significantly different from each other ($P < 0.05$).

Table 2. Dose-dependent effect of nimodipine on sperm quality in male rats

Treatment groups (n = 6)	Abnormal sperms (%)
Control	18.38 ± 3.57
Nimodipine 20 mg/kg	34.9 ± 4.89*[a]
Nimodipine 40 mg/kg	47.92 ± 6.03*[b]
Nimodipine 80 mg/kg	55.22 ± 7.73*[c]

Values represent mean ± SEM; n: number of animals in each group; *significantly different compared to control ($P < 0.05$); values with non-identical superscripts (a, b, c) within the same parameter are significantly different from each other ($P < 0.05$).

Histopathological findings of testicular tissue

Histological examination of the testes in the control rats shows normal structure, with clear components like seminiferous tubules and interstitial tissues; lumen of seminiferous tubules was filled with sperms (Fig.4A), in addition to the presence of mature spermatozoa heads and tails with mild differences in the size of tubules and no signs of inflammation. In group treated with 20 mg/kg of nimodipine, the morphology of testes was characterized by the presence of intratubular edema, absence of mature spermatozoa, preservation of spermatogonia and necrosis of tubular cells (Fig.4B). The magnitude of these changes was found to be dose-dependent, where at group treated with highest dose (80 mg/kg) revealed rise to marked absence of spermatogenesis and no presence of spermatogonia with marked increase in the intratubular edema and tubules were just ghost like shapes composed only of tubular outlines and few interstitial cells as shown in Figs.4D&E.

Histopathological findings of epididymal tissue

Histological images of control group showed that the epididymal epithelium enclosed a lumen containing spermatozoa, and the pseudostratified epithelium was composed of principle cells with nuclei at the base. In the nimodipine-treated groups, the epididymis (especially at 40 and 80 mg/kg doses) was filled with necrotic material and some of tubules were devoid of epithelium lining. Meanwhile, treatment with highest dose (80 mg/kg) nimodipine for 30 days showed more degenerative features including decrease in the epididymal wall thickness, loss of smooth muscle layer and absence of sperms in the lumen (Fig.4F).

DISCUSSION

It has been previously shown that CCBs reversibly impair reproductive functions in rats, where a study showed that exposure of male rats to CCBs resulted in a significant reduction in the epididymal sperm count and motility, which does not occur through inhibition of the pituitary-gonadal axis, because testosterone, follicle stimulating hormone and luteinizing hormones were unchanged [16]. In the present study, the result clearly demonstrates that treatment of male rats with nimodipine in different doses can seriously alter testicular structure and function. While the mechanisms of action of CCBs in terms of inhibition of calcium ion influx in the muscle cells of the heart and blood vessels are well documented [18], it is not known how precisely (especially for nimodipine) they contribute to the development of testicular dysfunction through the impairment of sperm motility and reduced sperm count. Nifedipine, verapamil and diltiazem have been previously shown to exert their sperm-immobilizing effect through calcium channel blockade [19]. Transmembrane fluxes of calcium ions are critically important for sperm-motility initiation and regulation [20].

In the present study, male rats administered different doses of nimodipine showed a significant decrease in the cauda epididymal sperm counts and activity, which can be explained on the basis of interference with calcium ion signaling required for sperm activity. Our present findings are in tune with many previous ones regarding male sterility caused by CCBs. Human sperms from hypertensive patients treated by nifidepine

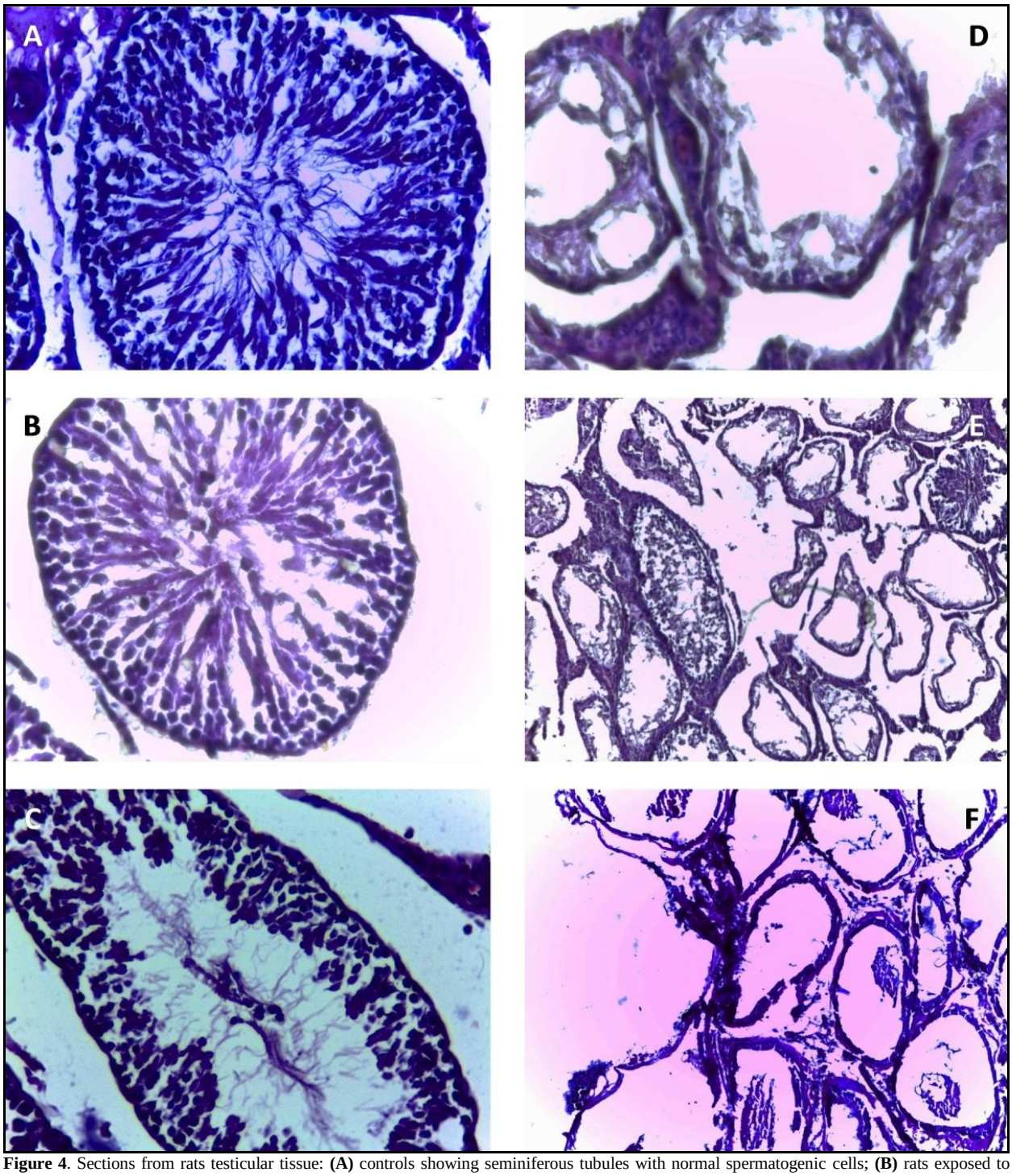

Figure 4. Sections from rats testicular tissue: **(A)** controls showing seminiferous tubules with normal spermatogenic cells; **(B)** rats exposed to 20 mg/kg nimodipine; **(C)** decreased spermatogenesis in group treated with 40 mg/kg nimodipine; **(D)** and **(E)** increase in degenerative changes and absence of spermatogenesis in group treated with 80 mg/kg nimodipine; **(F)** changes in epididymis of rats treated with 80 mg/kg nimodipine (H&E, x40).

failed to undergo acrosome reaction and *in vitro* fertilization due to a defect in the late stage of spermatogenesis [21, 22]. Most intriguingly, expression level of alpha-1H T-type Ca-channels in the testis of subjects with Sertoli-cell-only (SCO) syndrome, a condition of the testes in which only Sertoli cells line the seminiferous tubules thus azoospermic, is substantially lowered compared to the testis retrieved from normal subjects [23]. Moreover, subacute treatment of male rats with amlodipine (0.04 mg/rat/day for 30 days) lead to deleterious effects on the reproductive function, including decreased

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plasma follicle-stimulating hormone and testosterone, sperm density as well as the amount of mature spermatids and Sertoli cells counted in seminiferous tubule cross-sections [24].

The present study demonstrated that the nimodipine-induced changes in sperm count and motility was associated with alteration in the normal structure and morphology of the sperms, which might be attributed partly to the changes in intracellular calcium; previous reports indicated that well functioning calcium-homeostasis was associated with sperm movement, hyperactivation, acrosome reaction and fertilization [25]. While CCBs exerted their sperm-immobilizing effect through calcium channel blockade, they may alternatively act through oxidative stress-induced cytotoxicity as suggested by Anand *et al* [26]. Although there are contrary reports in the literature suggesting the anti-oxidative effects of CCBs, these reports were not related to the reproductive system [27]; accordingly, the oxidative stress mechanism in this respect cannot be excluded as reported by others [28].

Histological evaluation can be especially useful by providing a relatively sensitive indicator of damage, the nature of target cells and extent of toxicity, in addition to indicating the potential for recovery [29]. In the present study, there was significant and biologically meaningful histopathologic damage in excess of the level seen in the control tissues of the isolated reproductive organs, which could be considered an adverse reproductive effect. Since nimodipine disrupt the testicular histo-architecture, the Leydig cells, the only source of testosterone production, appeared to be affected; this might be associated later with the disruption of steroidogenesis in treated animals (unpublished data). Previously published results have clearly shown that long term administration of the CCB amlodipine causes a significant reduction in seminiferous tubular diameter and height of germinal epithelium [13]. The results presented here were compared favorably with previously published studies in which the investigators have reported that the anti-reproductive effects of CCBs were associated with structural changes, both in the sperms and reproductive tissues and these changes were reversible on discontinuation of therapy [21]. Hershlag *et al* [22], assessed sperm fertilizing potential in sperms from infertile male patients taking dihydropyridine type of CCBs as antihypertensive treatment; they reported subnormal expression of sperm head-directed mannose ligand receptors and failure of spontaneous acrosomal reaction in the sperms of such patients. The presented data indicated that the toxic effects of nimodipine on the reproductive system in male rats include both structural and functional defects and were dose-dependent; this finding was in tune with that reported by others, where toxic response on testicular tissues and sperm morphology and activity was correlated with the dose of other CCBs (nifedipine and ethosuximide) in mice [30].

In conclusion, for the first time we reported that nimodipine, in dose-dependent pattern, causes a significant drop in sperms structure and function associated with disarrangement of histological architecture of reproductive tissues of male rats.

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