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Effects of Amlodipine on Spermatological Parameters and Genital Tract Weight in Adult Wistar Male Rats*

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Abstract: Calcium ions are very important for many sperm functions involving sperm motility, reaction, and hyperactivation. The purpose of this study was to examine the effect of calcium channel blocker (CCB), Amlodipine (AML), on spermatological features, genital tract weight and spermatogenic cell density in adult male Wistar rats. Twelve rats (age, 12 weeks; weight, 290-350 g) were divided into two groups, six rats per each. Group I rats were fed normal diet without AML served as the control, Group II received 0.04 mg/daily AML for 30 days by oral gavage. Motility, left testis, right epididymis, prostate weight were decreased, and abnormal sperm rate was significantly (P<0.05) increased in AML groups compared with control group. The seminiferous tubular diameter and germinal cell layer thickness were not affected compared with control group. In conclusion, subacute administration of AML to male rats have detrimental effects on the spermatological parameters and genital tract weights, but not affect spermatogenic cell density of male rats.

Keywords: Amlodipine, Rat, Spermatologic parametres, Testis.

Yetişkin Wistar Erkek Ratlarda Amlodipinin Spermatolojik Parametreler ve Genital Organ Ağırlıkları Üzerine Etkileri

Öz: Kalsiyum iyonları, spermatozoon motilitesi, akrozom reaksiyonu ve hiperaktivasyon gibi pekçok spermatozoon fonksiyonu için çok önemlidir. Bu çalışmanın amacı kalsiyum kanal blokörü olan Amlodipinin yetişkin Wistar erkek ratlarda spermatolojik özellikler ve genital organ ağırlıkları ve spermatogenik hücre yoğunluğu üzerine etkilerini araştırmaktır.Yetişkin 12 erkek rat (12 haftalık yaşta, 290-350 gr ağırlıkta) her grupta 6 rat olacak şekilde iki çalışma grubuna ayrıldı. Grup I'de bulunan ratlar AML bulunmayan normal diyetle beslenip kontrol grubunu oluştururken Grup II'ye oral gavaj yolu ile 30 gün süreli 0.04 mg/gün dozunda AML verildi. Kontrol grubuna göre AML grubunda motilite, sol testis, sağ epididimis, prostat ağırlıklarında azalma, anormal spermatozoon oranında ise artış istatistiki açıdan (P<0.05) önemli bulunmuştur. Gruplar arasında seminifer tubul çapı ve germinal hücre duvar kalınlığı açısından istatistiki önem bulunmadı. Sonuç olarak subakut Amlodipin uygulamasının erkek ratlarda spermatolojik parameterler ve genital organ ağırlıkları üzerine olumsuz yönde etki yaptığı ancak spermatogenik hücre yoğunluğunu etkilemediği görüldü.

Anahtar Kelimeler: Amlodipin, Rat, Spermatolojik parametreler, Testis.

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INTRODUCTION

Calcium ions are present in the germ cells and directly included in the coordination of the following key processes that regulate or determine male fertility: blood testicular barrier (1), testosterone synthesis by Leydig cells (2), hormonal regulation of Sertoli cells function (3), secretory function of Sertoli cells (4), capacitation of sperm cells (5), sperm motility and acrosome reaction (6), spermatogenesis (3, 4), and penetration of oocytes by sperm cells (4, 6).

Calcium channel blockers (CCB), have been used commonly in the remedy of hypertension and angina. They block calcium inflow in vascular smooth muscle cells and reduce the blood pressure by decreasing peripheral vascular resistance (7).

Side effects on sperm fertilizing potential have also been based upon the beneficial practice of CCB (8), pointing to the possible male contraceptive agents. *In vitro* studies of human ejaculates incubated with diltiazem or verapamil inhibitors of Ca^{2+} intake, demonstrated reduced sperm motility and vitality, detrimental changes in the head and tail areas; curling of spermatozoa and restraint of acrosome reaction (9, 10).

Though, Morad et al. (11) showed that vascular specificity is extremely desired, many CCB (verapamil, diltiazem) may prevent hormonal secretion, involving the pituitary hormones. Moreover, reduced testosterone has been reported in rats that were given verapamil and diltiazem treatment (11).

Amlodipine, CCB belongs to dihydropyridine (DHP) group, has been indicated to reduce sperm count and motility in semen collected from cauda epididymis of rats (12, 13). The purpose of this study was to examine the effect of treatment of male rats with the AML on spermatological features and genital tract weight in adult male Wistar rats.

MATERIALS and METHODS

Chemicals

Amlodis (5 mg tablet, Amlodipine besylate) and cefamezin (1 g vial, 1000 mg cefazolin) were obtained from Zentiva (Turkey). Ketalar (10 ml vial, Ketamin HCl 50 mg/ml) was obtained from Pfizer (Turkey), and xylazine (25 ml vial each ml solution contains 20 mg xylazine base) was purchased from Bayer (Turkey). AML was liquefied in sterile isotonic solution (0.9% NaCl).

Animals and Experimental Design

Twelve male Wistar Albino rats (mean age, 12 weeks; weight, 290–350 g) were used in this study at the Afyon Kocatepe University Experimental Animal Research Center. The experimental orders were approved by the Animal Care and Use Committee at Afyon Kocatepe University (2016-49553702/09), and were by the National Institute of Health Guide for the Care and Use of Laboratory Animals. The animals were randomly allocated into two groups (n=6) and housed in a controlled environment (22°C; 12-hour light–dark cycle) and free access to food and water was allowable**.** Rats in group I (control group) were fed normal diet without AML, whereas those in group II received normal feed containing 0.04 mg/daily AML.

Epididymal Sperm Evaluation

Forward progressive sperm motility was examined under a phase contrast microscope with the heated stage as previously described by Sönmez et al. (14). The abnormal spermatozoa rates were assessed according to Watson (15). Briefly,, 10 µl semen sample was mixed with Giemsa stain, smeared and examined under phase contrast microscope (magnification 1000 x oil immersion). One hundred spermatozoa were counted for sperm morphology.

Histologic Examination

Testicular tissue was fixed in Bouin's solution, embedded in paraffin wax, sectioned at 5 µm thicknesses and stained with Mayer's hematoxylin and eosin (H&E). For the assessment of spermatogenic cells changes, ten seminiferous tubules (ST) were randomly evaluated per section. The diameters and germinal cell layer thicknesses (GCLT) (from the basal membrane to the lumen of the tubule) were calculated using an ocular micrometer with a light microscope. Accordingly, the mean size of ST and GCLT were determined.

Statistical Analysis

Statistical analysis was performed with SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA) software. Descriptive analyses were provided as mean ± Standard Error of the Mean (SEM) performed with the Student's *t*-test. The results were statistically significant when P was <0.05.

RESULTS

Sperm Motility and Abnormal Sperm Rate

Table 1 shows the sperm motility and abnormal sperm rate of the Group I and Group II. The percentage of sperm motility in Group I and Group II were 83.3±2.11% and 70.0±2.58%, respectively. The percentage of abnormal sperm rate of Group I and Group II were 11.5±1.33% and 18.7±1.33%, respectively. These results showed that sperm motility was significantly reduced and abnormal sperm rate was significantly increased in Group II (P<0.05).

Genital Tract Weight

Reproductive tract measurements are given in Table 1. The left testis, right epididymis and prostate weights of the Group I and Group II were 1.75±0.06 and 1.50±0.09, 0.63±0.02 and 0.5±0.03, 0.51±0.06 and 0.29±0.04 gm, respectively. The left testicle, right epididymis, and prostate weights were significantly decreased in Group II compared with Group I (P<0.05). However, right testis, left epididymis, bulbourethral and seminal vesicles' weights in the Group II were not significantly different when compared with the Group II.

Table 1. Mean (X±S.E.M) of spermatological parameters and genital organ weights in adult Wistar male rats.
Tablo 1. Yetişkin Wistar erkek ratlarda spermatolojik parametreler ve genital organ ağırlıklarının ortalaması (X±S.E.M.).

Group	Motility (%)	Abnormal Sperm Rate (%)	Right testis	Left testis	Right Epididymis	Left Epididymis	Bulbourethral	Prostate	Seminal vesicles
Control	83.3±2.11[a]	11.5±1.33[b]	1.70±0.06[a]	1.75±0.06[a]	0.63±0.02[a]	0.60±0.02[a]	0.60±0.06[a]	0.51±0.06[a]	1.05±0.06[a]
AML	70.0±2.58[b]	18.7±1.33[a]	1.50±0.11[a]	1.50±0.09[b]	0.50±0.03[b]	0.60±0.01[a]	0.40±0.03[a]	0.29±0.04[b]	0.90±0.08[a]

Values (mean ± S.E.M.) with different superscripts (a and b) within the same column were significant at P < 0.05., AML: Amlodipine.

Spermatogenic Cell Density

Spermatogenic cell density is given in Table 2. ST diameter with GCLT in the Group II was not

significantly ($P < 0.05$) different when compared with the Group I.

Table 2. Mean (±SEM) of the spermatogenic cell density in adult Wistar male rats (X ± S.E.M.).

Tablo 2. Yetişkin Wistar erkek ratlarda spermatolojik hücre yoğunluğu ortalaması (X ± S.E.M.).

Group	Diameter of ST (μm)	GCLT (μm)
Control	320.61±13.57[a]	131.43±8.90[a]
AML	286.60±11.96[a]	116.95±5.44[a]

Values (Mean ± S.E.M.) with different superscripts (a and b) within the same column showed significant differences ($P < 0.05$). AML: Amlodipine, ST: Seminiferous tubules, GCLT: Germinal cell layer thickness

DISCUSSION and CONCLUSION

AML has become the second drug of choice for hypertension, though its side effect on fertility has been proved to some extent. The exact mechanism of AML causing infertility in male remains to be completely elucidated. Therefore, the effects of AML on the spermatological features and genital tract weight in adult male Wistar rats have been explored in the present study.

In our study, it was indicated that rats treated with AML are more susceptible to a significant reduction in sperm motility and increase in abnormal sperm rate at a dose of 0.04 mg /kg/ day for 30 days. This finding is in agreement with the findings of many investigators. Almeida et al. (5) suggested that subacute administration of male rats with AML (0.04 mg/rat/day for 30 days) cause detrimental effects on the reproductive function, spermatological parameters as well as the number of mature spermatids. Akinlolu et al. (16) reported that administration of ≥ 15 mg/kg bodyweight AML besylate, to accelerate the relief of hypertension by drug abusers, could have adverse effects on testicular histology, sperm cells morphology, and motility. Oskouei et al. (17) reported a significant reduction in sperm motility, acrosome reaction and an increase in the number of dead sperm in Syrian male mice received AML at a dose of 2.5 mg /kg once daily for 30 days compared with control group. In a study reported by Dominic and Padjama (18) revealed a significant decrease in sperm count and motility when AML was used at a dose of 0.9 mg /kg once daily for 28, 42, 91 and 126 days in rats.

CCB causes a decrease in sperm density, motility and cellular energy content in guinea pigs (19). Chaloob et al. (20) reported a significant dose-dependent decrease in sperm motility and increase in sperm abnormalities when Nimodipine was used at the dose of 20, 40, 80 mg/kg once daily for 30 days in rats. In a different study a significant reduction in sperm motility was reported when Nifedipine was used at a dose of 0.571 mg /kg once daily for 30 days in rats [13]. Benoff et al. (21) suggested that Nifedipine produces reversible male infertility by changing the cholesterol content of the sperm. They also documented that the AML-induced change in sperm motility and abnormal sperm rate might be attributed partly to the changes in intracellular calcium. Previous data illustrated that well-functioning calcium homeostasis was related to sperm motility, hyperactivation, acrosome reaction and fertilization (22).

The assessment of genital tract weight of animals has a prominent role in male reproductive risk evaluation. Organs that are often evaluated involve the testis, epididymis, prostate, bulbourethral and seminal vesicle. In this study we observed that sacrificed rats given a dose of 0.04 mg /kg once daily for 30 days showed a reduction in the weight of left testis, right epididymis, and prostate. This finding similar to Karthick and Harisudla (23) who found a decrease in the weight of the testis when AML was used at a dose of 0.45 mg /kg once a daily for 30 days compared with control group.

Sperm cell density including ST diameter and GCLT were not significantly affected when compared with control group in the present study. Our results differed from those obtained by Latif et al. (4) who found long-term administration of the AML caused a significant reduction in ST diameter and GCLT. Marked changes were not observed in the histological structure of testis under short term treatment. These results indicated that the complete arrest might be noticed after long-term

administration for more than 64 days, the period needed by the spermatogonia to become mature spermatozoa.

The results of the present study emphasized the detrimental effects of subacute administration of AML on the reproductive function of male rats. Further researches are necessary to investigate the potential effects of long-term administration AML on sexual impairment a large number of rats.

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