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Research Article

HISTOLOGICAL AND HISTOMETRIC STUDY OF TESTIS IN ALBINO RATS TREATED WITH AMLODIPINE

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ABSTRACT

Amlodipine is the most common drug of choice to treat hypertension, one of its side effects is infertility and its effect on the testis of male albino rats is not well documented. **Aim:** To observe the effect of amlodipine in testis of male albino rats by the histological and histometric method. **Materials& Method:** we selected 12 adult male albino rats divided into 2 groups, group 1 treated as control group 2 treated as experiment and amlodipine is administered for 30 days. After 30 days testis were removed and analysed histologically and histometrically. **Result:** Though there are no marked changes, but early degenerative changes and reduction in weight of testis of experimental rats observed. **Conclusion**: Presence of vacuolated spermatogenic cells in some of the seminiferous tubules indicates early degeneration and arrest of spermatogenesis.

Keywords: Hypertension, Infertility, Amlodipine Side Effects.

INTRODUCTION

Hypertension is one of the leading causes of the global burden of disease. Approximately 7.6 million deaths (13–15% of the total) and 92 million disability-adjusted life years worldwide were attributable to high blood pressure in 2001.[1] Hypertension doubles the risk of cardiovascular diseases, including coronary heart disease (CHD), congestive heart failure (CHF), ischemic and hemorrhagic stroke, renal failure, and peripheral arterial disease.[1]The burden of hypertension increases with age and among individuals aged ≥ 60, its prevalence is 65.4%. Amlodipine has become the second drug of choice for hypertension[2], though its side effect on infertility has been proved to some extent.[3,4] The exact mechanism of amlodipine causing infertility in male remains to be completely elucidated moreover, its effect on the microscopic structure of the testis is not well documented histometrically, and therefore it has been planned to observe histological observation of testis, histometric analysis of testis, determine the weight of testis.

MATERIALS AND METHODS

A total of 12 adult male albino rats was obtained from the central animal house, Rajah Muthiah Medical College, Annamalai University, which were maintained under standard laboratory conditions at 28±2°C were provided with standard rat diet and water *ad libitum*. After getting ethical committee clearance, the animals were divided into 2 groups. Group I comprised of 6 animals; Control: received vehicle only (0.01% ethyl alcohol) and group II comprised of 6 animals; Experimental (Test group): received amlodipine orally (0.45mg/kg/day) given for 30 days. All the animals were sacrificed after 30 days

of the experimental period, the testis were removed, trimmed free of adipose tissue and connective tissue. The weight of the testis was recorded. The organs were fixed in Bouin's fluid for a total period of 24 hours. After fixation, the tissues were processed for light microscopy, the tissues were stained with Haematoxylin and Eosin and Masson's Trichrome stain for connective tissue. The stained sections of testis were examined in low power (x 100) and high power (x400). Qualitative evaluations of testicular sections were supplemented by the use of the semi quantitative testicular biopsy score count (TSBC) of Johnson (1970) to estimate the extent of testicular alterations. For histometric assessment the principles emphasized by Hans Elias and Pauly (1996) as well as Weibel and Hans Elias (1967) were strictly employed for estimating the volume and surface area of various tissue components. Volume of parenchyma and stroma were estimated by point count using the eyepiece reticule with low magnification. The formula used for estimation of volume (Vi = Pi / PT) Where Vi = volume of tissue component per unit volume of tissue, Pi = number of points touching the tissue component, PT = total number of points in the reticule. The height of the secretory epithelium and the diameter of tubules was measured using as ocular micrometer with high magnification.

Statistical Analysis**:** Using latest HPSS software

RESULTS

There is a decrease in weight of the testis of the experimental rats than the control rats presented in table 1. Histological observation of testis revealed there was no testicular alteration and the epithelium was intact with normal spermatogenesis from experimental animals, when compared with control animals. However, on closer examination under high power revealed an interesting finding in these test group animals.

Fig 1: Sections of testis Control and test rats (H &E 100X)

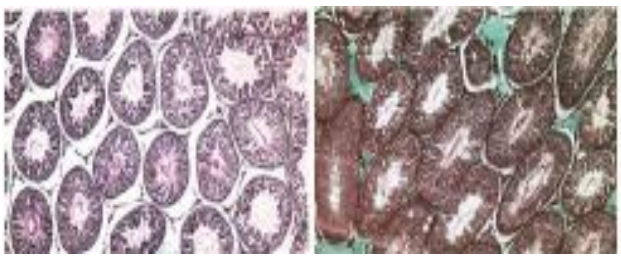

Fig 2: Testis control and test (Masson's trichrome stain 100X)

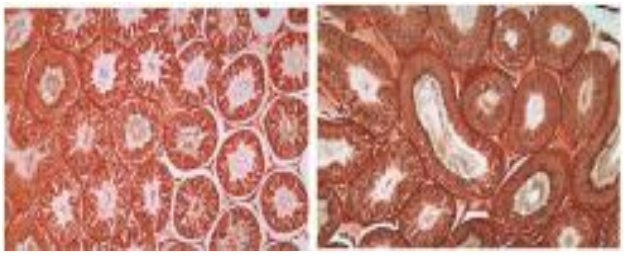

Fig 3: Sections of testis from control and test (Van Gieson's stain 100X)

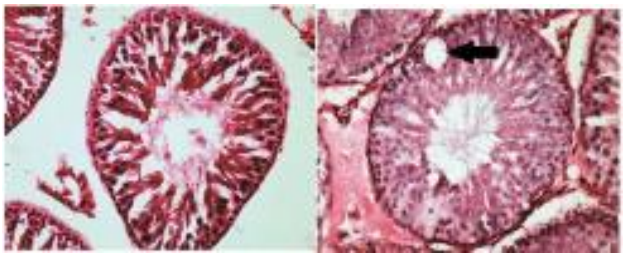

Fig 4: Sections of seminiferous tubules from control , test (H& E 400X)

Arrow (test) shows early fatty degeneration of spermatogenic cells

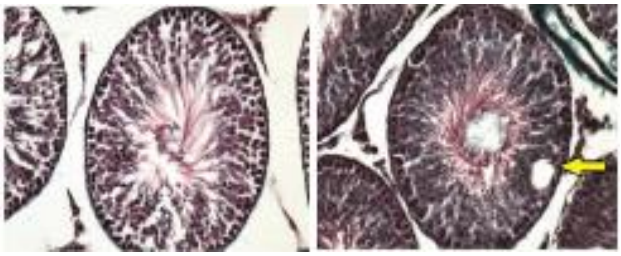

Fig 6: Seminiferous tubules from control, test (Masson's trichrome 400X)

Arrow (test) shows early fatty degeneration of spermatogenic cells

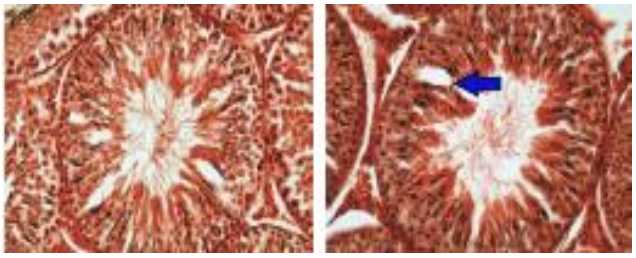

Fig 7 : Seminiferous tubules from control, test (Van Gieson's 400X)

Arrow in (test) shows early fatty degeneration of spermatogenic cells

Table 1 : Weight of testis, Volume of tissue components (values are expressed as Mean ± SEM)

Animal group	Testis (grams)	Volume of tissue components			Diameter of Seminiferous Tubules (µm)
		Seminiferous tubules (mm^3/mm^3)	Connective tissue (mm^3/mm^3)	Leydig cell (mm^3/mm^3)	
Control	1.2595	0.7747 ± 0.0216	0.1309 ± 0.0245	0.0719 ± 0.0075	279.64 ± 10.922*
Test group	0.9109	0.6764 ± 0.0233	0.2342 ± 0.0233	0.0867 ± 0.0087	268.45 ± 16.19*

* - $p < 0.05$

There was the presence of vacuolated spermatogenic cells interspersed among the seminiferous epithelium. (Fig 2). Histometric data of testicular tissue components are summarized in table 1. The quantitative analysis of various tissue components of the testis showed no significant change in any component. But the diameter of seminiferous tubules showed a significant increase in testis of experimental (Test group) animals when compared to those of control animals.

DISCUSSION

The anti-reproductive effect of amlodipine on male reproductive organs varies from decrease in weight of testis, epididymis, seminal vesicle and prostate, decrease in hormone levels of testosterone, FSH and LH, and partial / complete arrest of spermatogenesis by de-regulation of Ca2+ homeostasis, loss of libido and erectile dysfunction. In our present study, we observed that sacrificed rats after 30 days of treatment with amlodipine showed a reduction in the weight of testis. This finding is in agreement with the findings of many investigators. Rabia et al.[5] showed a significant drop in absolute testicular weight, gonado – somatic index and serum testosterone levels in rats after amlodipine treatment. Similar anti reproductive effects were described by Ayodele O et al., Benoffet al.[6,7]. They noticed altered serum parameters (reduction in sperm count & motility) The drug may not have a direct effect on Leydig cells, as the present study shows that Leydig cells are not affected histologically and histometrically in the treated animals. It appears that, the mode of action of this calcium channel blocker is through hypothalamo – hypophyseal – testicular axis by altering either the release of GnRH from hypothalamic neurons or the release of gonadotrophins from the pituitary, this can be augmented by the findings of Bourguignon JP, et al.[8] Who showed that in the presence of calcium channel blockers, the release of GnRH was marked and reversibly reduced. Lee JH et al [9]told nifidepine causes male infertility by deregulation of Ca2+ homeostasis in testis of mice and arrest of spermatogenesis. Juneja.R et al., Suresh C. Joshi et al.[10,11]also told calcium channel blocker causes decrease in sperm density, sperm motility and cellular energy content in guinea pigs. Histopathological findings exhibited partial arrest of spermatogenesis in experimental animals. With above findings, we carried the present work i.e degenerative changes occurring in the seminiferous epithelium indicate that the amlodipine causes partial arrest of spermatogenesis due to the deregulation of Ca2+ homeostasis. This partial arrest of spermatogenesis is due to degeneration of spermatogenic cells observed by us and is supported by reduction in weight of testis. Although marked changes were not observed in the histological structure of testis under low power, early degenerative changes were noticed in the seminiferous epithelium under high power this indicates the beginning of the arrest of spermatogenesis. Probably the complete arrest may be noticed after long term treatment for more than 64 days as the spermatogonia takes 64 days to become mature spermatozoa.

CONCLUSION

The following conclusions are arrived at from the findings of our study on effect of amlodipine on testis in albino rats. There is a marked decrease in weight of testis, which may be correlated to decrease in spermatogenesis as evidenced from the sparse content of the spermatozoa presence of vacuolated spermatogenic cells in some of the seminiferous tubules indicates early degeneration and arrest of spermatogenesis. Further the mode of action of the

drug is probably through hypothalamo – hypophyseal – testicular axis as the Leydig cells parameters are not disturbed in the experimental animals, and a long term study is planned to identify the effects caused by amlodipine.

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