# EFFECTS OF AMLODIPINE ON SERUM TESTOSTERONE, TESTICULAR WEIGHT AND GONADO-SOMATIC INDEX IN ADULT RATS

**Rabia Latif, Ghulam Mustafa Lodhi*, Muhammad Aslam****

**Department of Physiology, Army Medical College, Rawalpindi, *Department of Physiology, Islamic International Medical College, Rawalpindi, **Department of Physiology, Shifa College of Medicine, Islamabad, Pakistan**

**Background:** Calcium ions are vital in many biological processes including hormonal secretion, mitosis, reproduction, fertility and regulation of gene expression. Thus, calcium antagonists who are frequently prescribed for the cure of cardiovascular diseases may affect any of these physiological processes. **Methods:** This quasi experimental study was done at Army Medical College, Rawalpindi from October 2007 to March 2008. Thirty male Sprague Dawley rats, purchased from National Institute of Health, Islamabad were divided into group A (vehicle treated controls) and group B (amlodipine treated). After receiving drug treatment for 50 days, all the rats were sacrificed. Serum was stored to measure testosterone by enzyme immunoassay technique. The testes were removed for measuring absolute testicular weight and gonado-somatic index. **Results:** Serum testosterone, absolute testicular weight and gonado-somatic index were found to be significantly reduced in amlodipine treated rats. **Conclusion:** These results suggest that long term treatment with amlodipine might be associated with significant testicular regression and reduction in serum testosterone. Furthermore, since dihydopyridine antagonists are widely used in hypertension, our data may have some clinical implication in the management of infertility associated with hypertension.

**Keywords:** adverse effects, calcium channel blocker, amlodipine, testosterone, testis, gonado-somatic index

## INTRODUCTION

Calcium channel blockers (CCBs), the most frequently prescribed drugs for the cure of cardiovascular diseases, block transmembrane calcium influx through calcium channels. Calcium ions are vital in many biological processes including excitation-contraction coupling, excitation-secretion coupling, mitosis, fertilization and regulation of gene expression, but the effects of CCBs by and large remain confined to heart and vascular smooth muscles. This tissue specificity is because of heterogeneity of calcium channels or 'use-dependence' characteristic of CCBs, i.e., blocking more effectively those calcium channels which are most active.[1]

However, despite exhibiting substantial cardiovascular selectivity, reports exist for infertility in males who are using CCBs.[2,3] The exact mechanism of CCBs in causing infertility remains to be completely elucidated, thus, necessitating the need to study this area.

Testosterone is the principal male reproductive hormone and literature review reveals contradictory reports regarding the effects of CCBs on serum testosterone.[4,5] Moreover, keeping in mind antiproliferative effects of CCBs[6,7], it seems that CCBs may hamper ongoing proliferative activities of various testicular cells and interfere in reproduction. So present study was designed and carried out to observe the effects of amlodipine on serum testosterone and testicular growth in adult rats.

## MATERIAL AND METHODS

Thirty adult male sprague dawley rats (90 to 120 days old) weighing about 140 g, purchased from National Institute of Health, Islamabad, were maintained under standard laboratory conditions at 28±2 °C with constant light-dark cycle and were provided with standard rat diet and water *ad libitum*. Rats were divided equally into two groups, Group A (control) was administered vehicle (0.5 ml distilled water/rat/day) orally and group B (experimental) was given amlodipine dissolved in distilled water (0.14 mg/kg/0.5ml/rat/day) with the help of a gavage needle for fifty days.

All the animals were sacrificed 24 hours after the last dose following protocols and ethical procedures. Blood samples for hormone assay were collected from intracardiac sampling under deep ether anaesthesia. Serum was separated by centrifugation, frozen and stored at -80 °C until assayed. The testis were dissected out and weighed with Sartorious digital balance. Gonado-somatic index was determined with the help of formula:[8]

**Gonado-Somatic Index(GSI)=)Gonad weight/total body weight)×100**

where Gonad weight=(weight of the right testis+ weight of the left testis)/2

Enzyme immunoassay of serum testosterone was carried out using ADALTIS-EIAgen Testosterone Kit-LI 4011 K (Italy).

The data were processed statistically by using SPSS version 13. 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'.

The difference was considered significant if *p*-value was found less than 0.05.

## RESULTS

Amlodipine at the dose of 0.14 mg/kg body weight significantly decreased serum testosterone level. There was no significant difference in the body weight of the two groups in the end. However, absolute weight of the testes was significantly reduced ($p<0.05$). Gonado-somatic index (GSI), which is a better way to assess the damage to the testes in relation to the body, was also significantly reduced in experimental group.

**Table-1: Comparison of mean serum testosterone level, mean body weight, mean absolute weight of the testes and gonado-somatic index in control and experimental groups (n=15)**

| Parameters | Group-A (Control) (n* =15) | Group-B (Test) (n =15) | *p*-value** |
|---|---|---|---|
| Serum testosterone (ng/ ml) | 2.40±1.29 | 1.07±0.77 | 0.002 |
| Mean body weight at the end of experiment (g) | 289.07±15.48 | 280.33±7.67 | 0.064 |
| Absolute weight of the testes (g) | 1.47±0.159 | 1.27±0.220 | 0.007 |
| Gonado-somatic index | .512 | .452 | .039 |

Values are expressed as mean ± SD. *n=total number of rats used, ***p*-value represents the comparison between experimental and control groups. P value less than 0.05 is taken as significant.

## DISCUSSION

The study was deliberately conducted on a normotensive animal model with the intention of demarcation of the direct drug effects from vascular insufficiency inherent in hypertensive states. Throughout the experiment, all rats showed almost equal weight gain. Even the group B rats did not show statistically significant growth retardation or weight loss. This corresponds to the reports of no systemic toxicity observed with the use of calcium antagonists. This also confirms that although intracellular calcium ions play essential role in the regulation of cell growth, calcium channel blockers exhibit inhibitory influence on cell proliferation only in malignant cells and not in normal cells.[9]

However, this is contrary to the report of Mel'nikova and Timoshin[10] who have documented significant reduction in body weight with the use of verapamil. This variability might be explained on the basis of difference in the classes of CCBs being used in both studies.

Amlodipine caused a statistically significant reduction of serum testosterone level in experimental group. These observations are in agreement with other studies[4,11,12] reporting similar effects by the use of either amlodipine or other calcium antagonists on serum testosterone level. However, our observations are contradictory to the study conducted by Albers *et al*[5] in which they have shown that chronic use of Nifedipine which belongs to the same class as that of amlodipine, that is dihydropyridine class, is not associated with testosterone suppression. This variation might be because of differences in the weight of experimental animals, the type of drug used the route of administration of drug or the time duration for which the drug was administered.

The reduction in serum testosterone levels by amlodipine indicates either a direct effect of drug at Leydig cell level or an indirect effect by disturbing the hormonal milieu at hypothalamo-pituitary axis. Bourguignon *et al.*, showed that in the presence of calcium channel blockers, the release of GnRH from hypothalamic neurons was markedly and reversibly reduced.[13] GnRH induced LH secretion from pituitary gonadotrophs is also calcium dependent because the induction/repression of the LHß gene is dependent on calcium influx.[14]

The weight of the testis is one of the markers of a possible alteration in androgen status. A decrease in testicular weight and GSI (a better way to assess the damage to the testes in relation to the body) in experimental rats is most likely due to decreased level of serum testosterone, as androgen exerts its major role in sex organs.[15]

One of the causes of decrease absolute testicular weight and GSI may be an effect of poor nutrition or decrease food intake.[16,17] Since both the groups were supplied with the same standard rat diet and their food intake was also satisfactory, there is less likelihood of poor nutrition being the cause of decrease testicular weight in experimental group.

Another cause of drop in the weight of the testes and GSI that needs to be explored is suppressed spermatogenesis. It has been shown that a strong correlation exists between testis weight and function (spermatogenesis) in mammals. In the absence of any known pathology, testis weight is highly related to daily sperm production.[18] Decrease sperm density by the use of amlodipine, as already shown by Almaida *et al*[4] strongly favors the suppressed spermatogenesis being the cause of decrease GSI.

Last but not the least, literature review strongly points towards the importance of pituitaries in maintaining testisular size and weight. A sudden increase of rat serum gonadotropins at the time of birth is followed by a rapid increase of absolute and relative testicular weights.[19] Pituitary FSH has been shown to increase the testicular size.[20] Almaida *et al* [4] have reported significant reduction in serum FSH level in amlodipine treated rats. Hence, lack of pituitary FSH can serve as a reason of decrease

absolute testicular weight and GSI in amlodipine treated group.

## CONCLUSION

Testicular histology and serum GnRH, LH and FSH levels can further help in arriving at a clear-cut conclusion. At this stage, the only conclusion is that the amlodipine causes a significant drop in absolute testicular weight, GSI and serum testosterone levels. Whether the reduction in absolute testicular weight and GSI has been caused by suppressed testosterone levels or *vice versa*, that needs to be further determined.

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**Address for Correspondence:**
**Dr. Rabia Latif,** House No. 25, Askari-VII, Adiala Road, Rawalpindi, Phone: +92-51-5573887, 0300-5184080
**Email:** rabialatif08@hotmail.com