Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 4 (2)

Amlodipine Besylate Administration(s) resulted in Skeletal and Testicular Dysmorphologies; and reduced Serum Testosterone in Adult Male Wistar Rats.

Akinlolu[1], A., Ghazal[1], L., Lewu[1], K., Babatope[2], F., Oluwaseun[2], I. and Adefule[2]A.

[1]Department of Anatomy, University of Ilorin, Ilorin, Kwara State.
[2]Department of Anatomy, Olabisi Onabanjo University, Ago-Iwoye, Ogun State.

Corresponding Author: Adelaja Akinlolu
E-mail: a3akin@gmail.com, akinlolu.aa@unilorin.edu.ng,
Tel: +2348062765308

ABSTRACT

Amlodipine Besylate is a long-acting calcium channel blocker used in Man for the treatment of hypertension and angina. The treatment regimen is 5 or 10mg daily. This study tested the hypothesis that prolonged oral administration of Amlodipine Besylate impairs fertility in adult male wistar rats. Twenty - nine normotensive adult male wistar rats (150 - 250g) were employed in the study: Rats of Control Group I (comprising of four rats) received 4mls of Normal Saline while rats of Experimental Groups II – VI (each comprising of five rats) received 5, 10, 15, 20 and 40mg/kg BW of Amlodipine Besylate respectively for 56 - 65 days. Gross morphology evaluations showed reduced bodyweight, hair loss, ataxia, dysmorphology and paralyses of limbs, vertebral anomalies and loss of movements in rats of Groups IV - VI in weeks 4 – 9 in a dose-dependent manner. No adverse effects were observed on testosterone levels in rats treated with Normal Saline, 5, 10 and 15mg/kg bodyweight of Amlodipine Besylate. However, significantly reduced serum testosterone was observed in treated with 20mg/kg bodyweight of Amlodipine Besylate. Histological analyses showed dose – dependent anomalies of the testes such as scanty and destroyed seminiferous tubules, wider tubular lumen and significantly reduced sperm cells in rats that received 20 and 40mg/kg BW of Amlodipine Besylate. This study observed skeletal dysmorphology, anomalies of behavioural functions and impaired fertility in adult male rats treated with 15mg/kg BW and higher doses of Amlodipine Besylate.

Key words: Amlodipine Besylate, Serum Testosterone, Adult Male Wistar Rats.

INTRODUCTION

The hypertrophy of cardiac muscles occurs in hypertension when the resistance against which the left ventricle must pump becomes elevated over a long period. Such resistance could be due to arteriosclerosis, thickening of intima media of arteries, hypertrophy of smooth muscles, reduced compliance of arterial walls and vascular stenosis[1-3]. Similarly, angina could result from focal coronary artery spasm and/or coronary vasoconstriction[1-3]. These cardiovascular anomalies in hypertension and angina are directly related to excitation-contraction coupling and contractions of vascular smooth muscles[1-3].

Increased intracellular entry of calcium ion results in formation of calcium – calmodulin complex, phosphorylation of myosin light chain, activation of myosin light chain kinase (MLCK) and contractions of vascular smooth muscles[1-4]. Relaxation of vascular smooth muscles, however, occurs following the release of calcium ions and activation of myosin phosphatase which splits the phosphate from the myosin light chain[1-4].

Amlodipine Besylate is a calcium ion antagonist which inhibits trans-membrane influx of calcium ion into cardiac and vascular smooth muscles. It inhibits calcium intracellular entry through voltage gated channels and activation of the actin-myosin complex leading to muscular relaxation. [1-3] It relieves ischemic burden through dilation of peripheral arterioles and reduced total peripheral resistance resulting in declined stress of the left ventricular wall, reduced myocardial energy consumption, reduced oxygen requirements, direct relaxant effects on vascular smooth muscles and reduced blood pressure[1-3].

In angina, Amlodipine Besylate relieves coronary artery spasm and vasoconstriction through dilation of peripheral arterioles and reduced total peripheral resistance resulting in increased blood flow to the heart muscle and increased myocardial oxygen delivery[1-3].
Calcium ions have ubiquitous presence in somatic and germ cells [5,6]. Calcium ions are, therefore, directly involved in the regulation of the following key processes that regulate or determine male fertility: blood testicular barrier [7,8,] testosterone synthesis by

Leydig cells[9], hormonal regulation of Sertoli cells function[10], secretion of products by Sertoli cells[5,6], capacitation of sperm cells[11], sperm motility[3], spermatogenesis[6,10,12,] penetration of oocytes by sperm cells, prevention of polyspermy and gene expression[3,5,12]. However, increased intrasperm concentration of calcium ions was determined to correlate negatively with sperm viability[13].

Hypertension is a global health concern which affects all races, sexes and different age groups; though it is more prevalent in the adults. It affects 20 percent of people living in the world, a third of which are unaware of their condition. Hypertension is usually treated with calcium channel blockers[1-3]. There have been contradictory observations concerning the effects of calcium channel blockers on testosterone concentrations[5] while concerns over the possible adverse effects of calcium channel blockers on male fertility remains[9]. Furthermore, individuals who abuse Amlodipine Besylate in Nigeria for the purpose of getting quick relief are more likely to take higher doses of the drug against medical prescriptions. For further considerations of the possible effects of Amlodipine Besylate as a calcium channel blocker on male fertility; this study evaluated the toxicity effects of the administrations of 5mg and higher doses of Amlodipine Besylate on fertility in adult male wistar rats.

MATERIALS AND METHODS

Ethical approval was sought and received from the Department of Anatomy of the University of Ilorin, Ilorin, Kwara State, Nigeria. The protocols for the use of animals in scientific research were strictly adhered to in compliance with World Health Organization's provisions.

Animal Care and Feeding

Twenty - nine normotensive adult male wistar rats weighing 150 - 250g obtained from the colony breed of the animal house of the Department of Anatomy, Obafemi Awolowo University, Ile-Ife, Osun State and the Department of Veterinary Physiology, University of Ibadan, Oyo State, Nigeria were employed in the study. The rats aged ten (10) to twelve (12) weeks. They were housed in individual cages in a well ventilated and fumigated room with ambient temperature and good lighting (12 hours of light and 12 hours of darkness). All rats were fed with standard pellet diet (Sesco Feeds Ikenne, Ogun State, Nigeria) and received water ad libitum. The rats were acclimatized for seven days before the start of experimental procedures. The weight of each rat was taken daily. Furthermore, each rat was examined daily for possible behavioural and gross morphological or physical changes.

Administrations of Drugs

Rats of Control Groups I (comprising of four rats) received daily oral administration of 4 millilitres of Normal Saline. In an adult 70kg (M), the treatment regimen of Amlodipine Besylate for the treatment of hypertension or angina is 5 or 10mg daily. 5mg Amlodipine Besylate (NORVASC®) was dissolved in 333mls of Normal Saline effectively without any residue. The rats of Experimental Groups II – VI (each comprising of five rats) received daily corresponding oral administration of 5, 10, 15, 20 and 40mg/kg Bodyweight of Amlodipine Besylate respectively for 56 - 65 days. Oral administration of drugs was done (with the use of a 5ml syringe and a flexible feeding tube long enough to reach the stomach through the oesophagus) by gastric gavage.

.

The (average) weight of rats employed in the study was determined as 200±5g. In an adult 70kg (M), the treatment regimen of Amlodipine Besylate is 5 or 10mg daily. Therefore, to determine the amounts of Amlodipine Besylate to be admnistered to each rat, the corresponding dosage (Xmg) for a 200g rat was calculated as follows: Xmg = (200g x 5mg)/70,000g = 0.014mg of Amlodipine Besylate.

If 5mg Amlodipine Besylate was dissolved in 333 milliltres of Normal Saline solution, the volume (X milliltres) of the Amlodipine Besylate/Normal Saline solution that would contain 0.014mg of Amlodipine Besylate was determined as follows: X milliltres = (0.014mg x 333 millilitres)/5mg = 4.662 millilitres/5 = 0.93 millitres (approximately 1 millilitres (mls) of Amlodipine Besylate/Normal Saline solution.

Therefore, rats of experimental groups II – VI received 1, 2, 3, 4 and 8mls of Amlodipine Besylate/Normal Saline solution as corresponding doses of 5, 10, 15, 20 and 40mg/kg bodyweight of Amlodipine Besylate respectively. Volumes of drugs solutions that were more than 2 mls were given two or four times daily to rats of Groups IV, V and VI for eased ingestion. This was in consideration of the maximum 3.4mls volume capacity of the stomach of adult rats[14] and the more likelihood of individuals who abuse Amlodipine Besylate to take doses of the drug two or more times daily. Rats of the Control Group I correspondingly received 4 mls of Normal Saline solution.

Excision and Fixation of the Testes and Epididymis

At the end of experimental procedure, each rat was sacrificed by cervical dislocation and the scrotal sacs opened. The whole testis was removed, taken out and fixed in 10% formal saline of at least five times its volume. The caudal epididymis was equally removed and put in Normal Saline of at least five times its volume. The testes and epididymis were put in separate containers and labeled appropriately.

Histological Analyses of the Testes

After complete fixation of the testes, blocks were embedded in paraffin wax and sections cut at 5µm (micron). The tissue sections were stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examination of the sections was

then carried out under the Olympus light microscope to determine possible cytoarchitectural changes of the testes following administrations of drugs.

Evaluations of Testosterone Concentrations
For testosterone analyses, the rats were dissected in the antero-median plane to expose the chest, the abdomen and pelvic region immediately after cervical dislocation. Blood samples for testosterone assays were obtained from the heart through the ventricles with the aid of a 5ml syringe from all rats employed in the study. The serum was separated by centrifugation (at 6000 revolutions per minute) and the serum testosterone concentrations determined based on the principle described below.

The Micro well Testosterone ELA is a solid-phase enzyme immunoassay which utilizes the competitive binding principle. Testosterone present in the serum competed with enzyme-labeled testosterone for binding with anti-testosterone antibody immobilized on the micro well surface. The amount of conjugate that bound to the micro well surface decreased in proportion to the concentration of testosterone in the serum. The unbound sample and conjugate were then removed by washing and the color development reagents (substrates) were added. Upon exposure to the bound enzyme, a color change took place. The intensity of the color reflected the amount of bound enzyme-testosterone conjugate and was inversely proportional to the concentration of testosterone in the serum within the dynamic range of the assay. After stopping the reaction the resulting color was measured using a spectrophotometer at 450 nm; and the testosterone concentration in the serum sample and concurrently run controls were determined from the standard curve.

RESULTS
Changes in Bodyweight (g) of Rats of Control and Experimental Groups During Experimental Procedure.
Rats of the Control Group I had significant increased bodyweight throughout the nine weeks of experimental procedures. (Table 1). In contrast, there was a drastic reduction in bodyweight of rats in Experimental Groups IV - VI during the first three weeks of administration. At the beginning of the fourth week, rats of Groups IV – VI experienced relative increased bodyweight till the end of experimental procedures. The gained increase in bodyweight could, however, not compensate for the previous weight loss since the rats were unable to attain the individual bodyweight recorded at the beginning of experimental procedure. (Table 1).

Table 1: Changes in Bodyweight (g) of Rats of Control and Experimental Groups During Experimental Procedure. ($P \leq 0.05$)

GROUPS OF RATS	WEEK 1 OF EXPERIMENTAL PROCEDURE	WEEK 5 OF EXPERIMENTAL PROCEDURE	WEEK 9 OF EXPERIMENTAL PROCEDURE	STATISTICAL SIGNIFICANCE AT
I	136.10g ± 3.57	139.10g ± 3.70	146.00g ± 3.40	YES
IV	198.00g ± 24.70	174.60g ± 19.30	177.80g ± 22.60	YES
V	160.00g ± 16.10		147.00g ± 13.60	YES
VI	187.60g ± 7.25	167.80g ± 16.20	171.10g ± 11.50	YES

Behavioural and Gross Morphological Changes Observed During Experimental Procedure:
No anomalies of gross morphology and behavioural activities were observed in rats of Control Group I. (Figure 1a). However, loss of hair around the tail, thigh, stomach, back and neck regions were observed in rats of Experimental Group V – VI in a dose – dependent manner. (Figures 1b - d). Severe deformities of the limb (Figures 1c – d) and vertebral column were observed in some rats of Experimental Group VI during weeks 3 – 9 of experimental procedure. This was accompanied with ataxia and loss of locomotion in affected rats. The limbs appeared swollen initially for about a week after which the limbs were lost due to paralyses. The lumbar or cervical region appeared bent or twisted in affected rats making them to become restless and unable to initiate movements.

No anomalies of gross morphology of the testes and epididymis were observed in rats of Control Group I and Experimental Groups II – VI when they were dissected and removed for histological and sperm viability analysis.

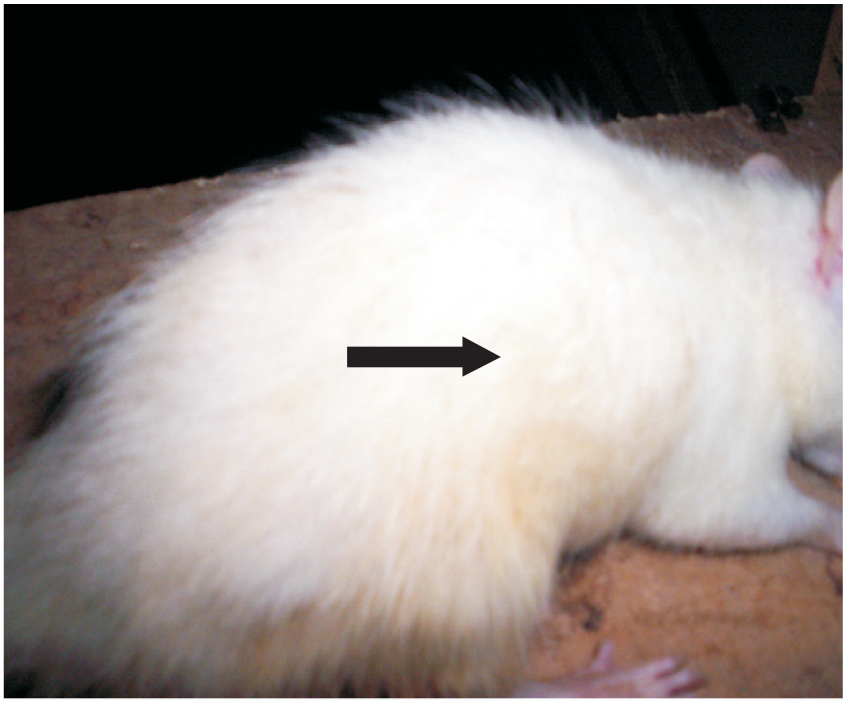

Figure 1a: Picture showing normal gross morphology in a rat of Control Group 1 which received 4mls of Normal Saline. The solid black arrow points to the hair and skin of the rat. No hair loss or skeletal deformity was observed.

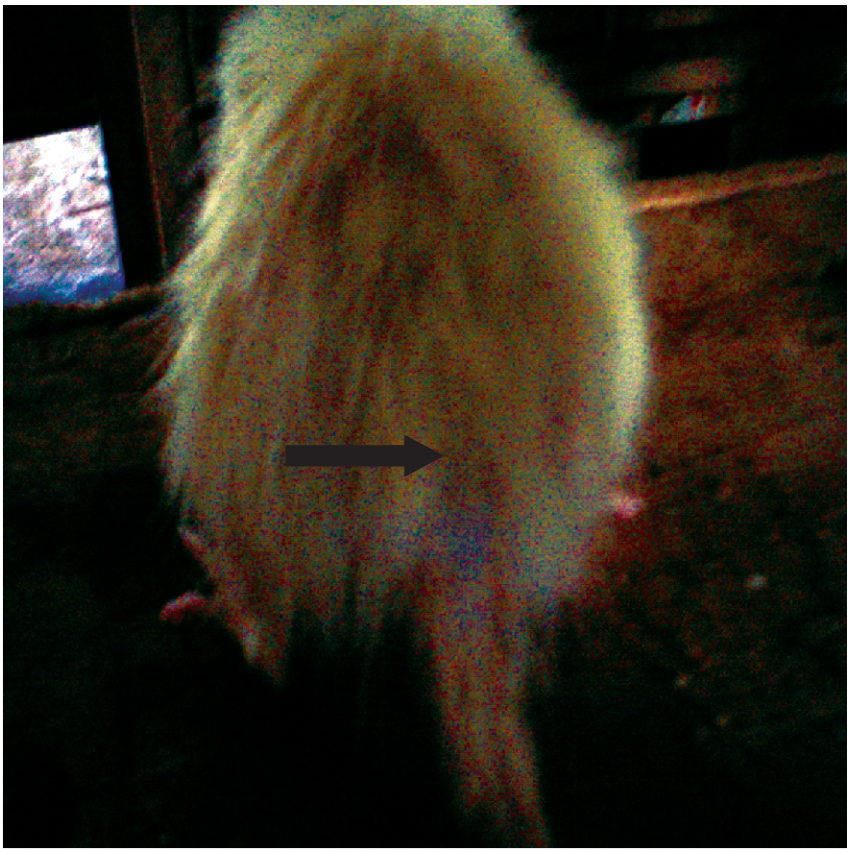

Figure 1b: Picture showing few loss of hair at the tail region of a rat of Experimental Group V which received 20mg/kg Bodyweight of Amlodipine Besylate. The solid black arrow points to the site of hair loss at the tail region of the rat. No skeletal deformity was observed.

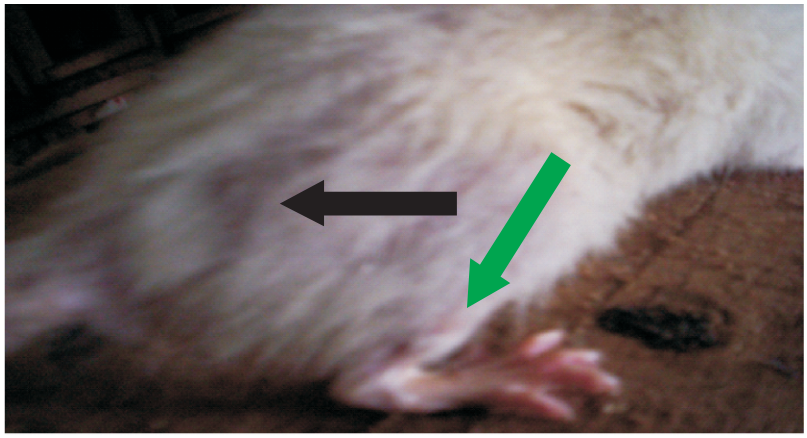

Figure 1c: Picture showing few loss of hair at the tail region of a rat of Experimental Group VI which received 40mg/kg Bodyweight of Amlodipine Besylate. The solid black arrow points to the site of hair loss at the tail region of the rat. The solid green arrow points to a deformed and paralyzed right limb of the rat.

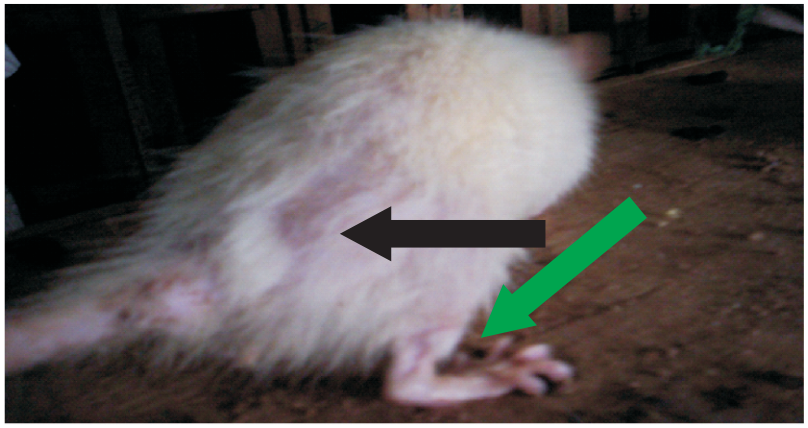

Figure 1d: Picture showing loss of hair at the tail region of a rat of Experimental Group VI which received 40mg/kg Bodyweight of Amlodipine Besylate. ***The solid black arrow points to the site of hair loss at the tail region of the rat. The solid green arrow points to a deformed and paralyzed right limb of the rat.***

Histological Analysis of the Testes in Rats of Control and Experimental Groups

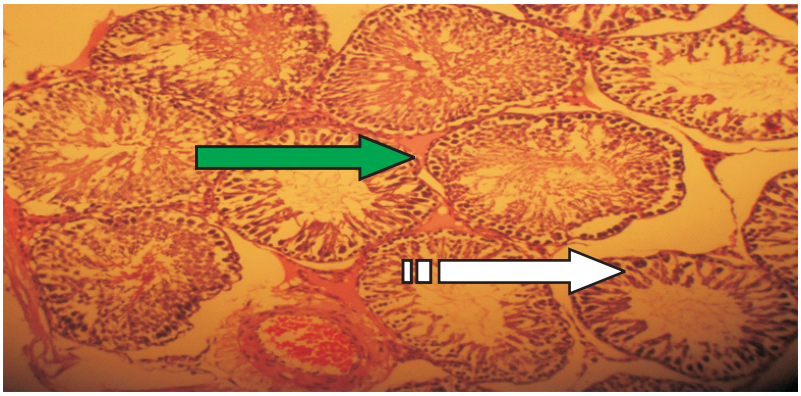

Figure 2a: Photomicrograph sample of the testes of rats of Control Group I which received 4mls of Normal Saline. Haematoxylin and Eosin X 100. Solid Green Arrow = Seminiferous Tubule; Broken Arrow = Lumen of Seminiferous Tubule. ***The cytoarchitectural components of the testis appear normal. There is adequate quantity of sperm cells in the lumina of the seminiferous tubules.***

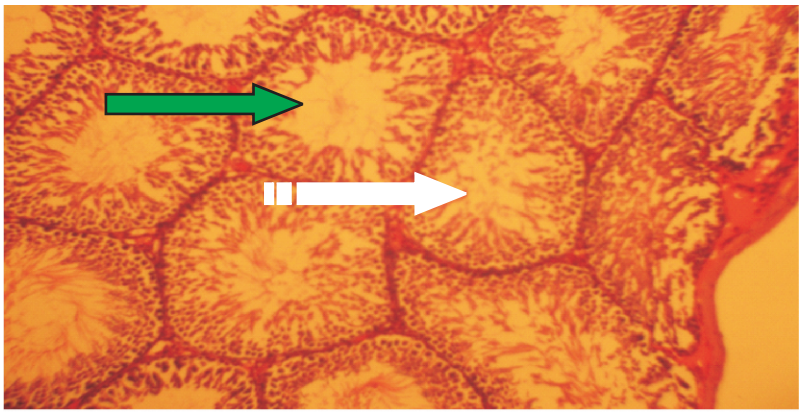

Figure 2b: Photomicrograph sample of the testes of rats of Experimental Group IV which received15mg/kg Bodyweight of Amlodipine Besylate. Haematoxylin and Eosin X 100. Solid Green Arrow = Seminiferous Tubule; Broken Arrow = Lumen of Seminiferous Tubule. ***The cytoarchitectural components of the testis appear normal. There is adequate quantity of sperm cells in the lumina of the seminiferous tubules.***

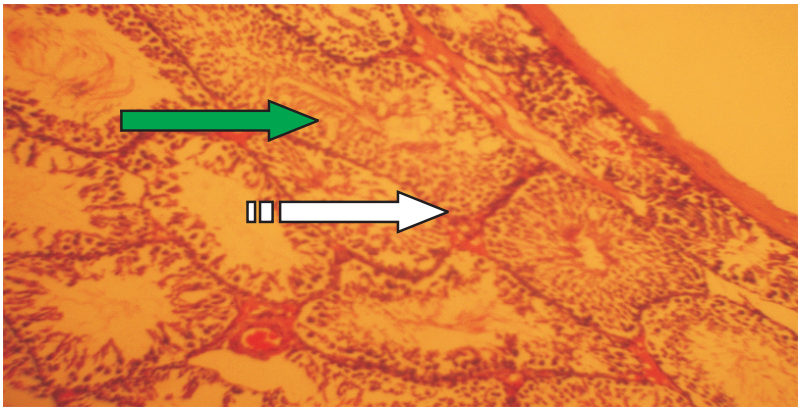

Figure 2c: Photomicrograph sample of the testes of rats of Experimental Group V which received 20mg/kg Bodyweight of Amlodipine Besylate. Haematoxylin and Eosin X 100. Solid Green Arrow = Seminiferous Tubule; Broken Arrow = Lumen of Seminiferous Tubule. ***The cytoarchitectural components of the testis appear normal. Reduced quantity of sperm cells in some lumina of seminiferous tubules was observed.***

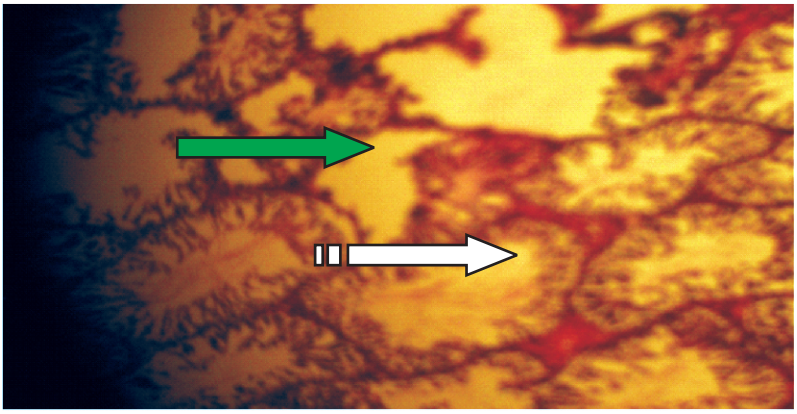

Figure 2d: Photomicrograph sample of the testes of rats of Experimental Group VI which received 40mg/kg Bodyweight of Amlodipine Besylate. Haematoxylin and Eosin X 400. Solid Green Arrow = Seminiferous Tubule; Broken Arrow = Lumen of Seminiferous Tubule. ***The cytoarchitectural components of the testis appear disrupted. There is marked reduction in the quantities of seminiferous tubules and sperm cells are absent in some lumina. Analyses of Serum Testosterone Concentrations***

Table 2: Analyses of Serum Testosterone Concentrations in Rats of Control and Experimental Groups.

DOSES OF DRUGS	TESTOSTERONE VALUE (ng/ml)
Normal Saline	0.40
5mg/kg/bw Amlodipine Besylate	0.50
10mg/kg/bw Amlodipine Besylate	0.50
15mg/kg/bw Amlodipine Besylate	1.20
20mg/kg/bw Amlodipine Besylate	0.22
40mg/kg/bw Amlodipine Besylate	0.1

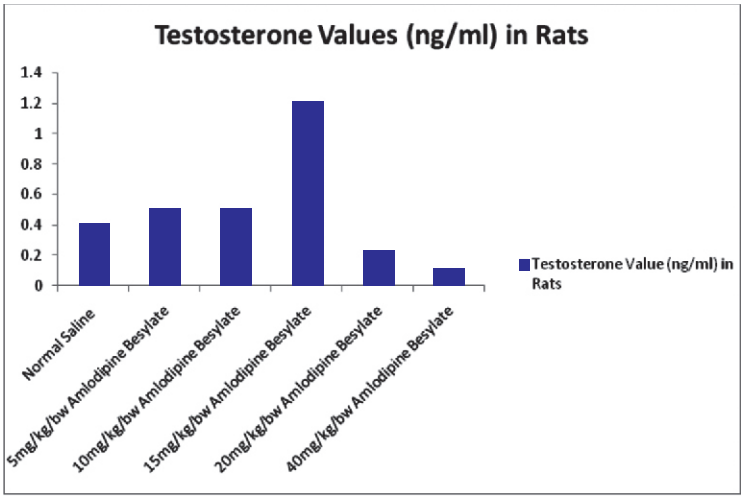

Figure 3: Graphical Analyses of Serum Testosterone Concentrations in Rats of Control and Experimental Groups.

DISCUSSION

Calcium ions have ubiquitous presence in somatic and germ cells[5,6]. Calcium ions are, therefore, of relevance in many biological processes such as maintenance of the integrity of cell membranes[1]; regulations of the permeability of cell membranes and cell adhesion1; mitosis and maintenance of body homeostasis[1,3]; excitation-contraction coupling and excitation-secretion coupling[5,6]; impulse generation in the heart1; signal transduction pathways where it is used as first and second messengers[15,16]; intracellular messenger for hormones, autacoids and transmitters[1]; controls of excitability of nerves and muscles, and the release of neurotransmitters during synaptic transmission[1,3,16]; bone formation, blood coagulation and co-factor for enzymes[1,3]; spermatogenesis[6,10,12]; fertilization and gene expression[5,12].

Rats of the Control Group I that received Normal Saline had significant increased bodyweight throughout the nine weeks of experimental procedure. (Table 1). Furthermore, no dysmorphology of body organs and anomalies of behavioural functions were observed in rats of Control Group I. (Figure 1a). In contrast, there was a drastic reduction in bodyweight of rats of Experimental Groups IV - VI that respectively received 15, 20 and 40mg/kg bodyweight of Amlodipine Besylate during the first three weeks of administration; which was not compensated for by subsequent gained increase in bodyweight during the fourth week of experimental procedure. (Table 1). The affected rats were unable to attain the individual bodyweight recorded at the beginning of experimental procedure. (Table 1). Rats of Experimental Group V – VI suffered loss of hair around the tail, thigh, stomach, back and neck regions in a dose – dependent manner. (Figures 1b - d). Severe deformities of the limb (Figures 1c – d) and vertebral column were observed in some rats of Experimental Group VI during weeks 3 – 9 of experimental procedure. This was accompanied with ataxia, limb paralysis and loss of locomotion in affected rats.]

Calcium ions have ubiquitous presence in somatic cells[5,6]; and make up about 2% (1 – 1.5kg) of the bodyweight in an adult. Calcium is the major component of bones and skeletal structures and more than 99% of calcium is stored in bones[1-3]. The observed reduced bodyweight, loss of hair, ataxia, loss of locomotion and vertebral anomalies in rats of Experimental Groups IV – VI could probably have resulted from the adverse effects of Amlodipine Besylate as a calcium channel blocker on intracellular entry of calcium ions in the skin, cerebellum (which coordinates equilibrium and balance), bones and other body organs.

The observed adverse effects of Amlodipine Besylate on skeletal morphology could be of considerations in older hypertensive women who may be susceptible to osteoporosis. This is in agreement with a previous study which opined that Amlodipine unlike Clinidipine did not prevent or ameliorate osteoporosis in ovariectomized hypertensive rats[17]. The regulatory functions of calcium in the controls of excitability of nerves and muscles, and the release of neurotransmitters during synaptic transmission[1,3,16] could have possibly been impaired which resulted in limb paralyses in the affected rats.

The cytoarchitectural components of the testis were normal with adequate quantity of sperm cells in the lumina of seminiferous tubules of rats of Control Group I. (Figure 2a). In contrast, cytoarchitectural components of the testis were disrupted with progressive or marked reduction in the quantities of seminiferous tubules in rats that received 20 or 40mg/kg bodyweight of Amlodipine Besylate. Some seminiferous tubules were destroyed while some seminiferous tubules have wider tubular lumina with few or no sperm cells in their lumina in rats that received 40mg/kg bodyweight of Amlodipine Besylate. (Figures 2b – d).

Similarly, there was significantly reduced testosterone concentration in rats that received 20 or 40mg/kg bodyweight of Amlodipine Besylate. However, normal testosterone concentrations were observed in rats that received Normal Saline (Control Group I), 5 and 10mg/kg bodyweight of Amlodipine Besylate. (Table 2 and Figure 3). Significantly increased testosterone concentration was observed in rats that received 15mg/kg bodyweight of Amlodipine Besylate, though the mechanism underlying this observation could not be determined in this study. (Table 2 and Figure 3).

The roles of calcium ions in the regulation of testosterone synthesis by Leydig cells[9], hormonal control of Sertoli cells function10, secretion of products by Sertoli cells[5,6] and spermatogenesis[3,10] could possibly have been impaired by the calcium ions blocking functions of Amlodipine Besylate which led to the observed adverse effects on the microscopic anatomy of the testes and testosterone concentrations in rats that received 20 or 40mg/kg bodyweight of Amlodipine Besylate.

Testosterone hormone plays regulatory roles on male puberty and spermatogenesis[3]. Therefore, reduced testosterone concentrations in rats that received 20 or 40mg/kg bodyweight of Amlodipine Besylate could possibly have been responsible for the significantly reduced quantity of functioning seminiferous tubules in rats treated with 20 or 40mg/kg bodyweight of Amlodipine Besylate This is in agreement with previous studies that observed reduced steroidogenesis[9], reduced serum testosterone[5,11], reduced height of germinal epithelium and significantly reduced seminiferous tubular diameter6 and suppressed spermatogenesis[6,12] in rats treated with Amlodipine Besylate.

This study observed anomalies of behavioural functions, skeletal dysmorphology and paralyses of limbs, reduced seminiferous tubules with wider tubular lumina coupled with reduction in serum testosterone in adult male wistar rats treated with 20 or 40mg/kg bodyweight of Amlodipine Besylate. This could imply that 5 - 10mg/kg bodyweight daily usage of Amlodipine Besylate in Man is probably safe. However, administration of 15mg/kg bodyweight or higher doses of Amlodipine Besylate in the treatment of hypertension could result in adverse effects on skeletal morphology, behavioural functions and in particular male fertility.

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology; 5th Edition, Jaypee Brothers Medical Publishers (P) Limited, India. 2003: pgs 297 – 506.
2. Nicholas AB, Nicki RC, Brian RW, John AA. Hunter. Davidson's Principles and Practice of Medicine. 20th Edition, Churchill Livingstone, Elsevier Limited, USA. 2006: pgs 496-585.
3. Guyton A, Hall J. Textbook of Medical Physiology, 12th Edition; Saunders, Elsevier Limited, USA. 2011: pgs 92 – 978.
4. Aguilar HN, Xiao S, Knoll AH, Yuan X. Physiological pathways and molecular mechanisms regulating uterine contractility. Hum. Reprod. Update. 2010; 16 (6): 725 – 744.
5. Latif R, Ghulam ML, Muhammad A. Effects of Amlodipine Besylate on serum testosterone, testicular weight and gonado-somatic index in adult rats. J Ayub Med Coll Abbottabad. 2008; 20(4).
6. Latif R, Muhammad A, Mazhar HM, Idrees FB, Azhar M, Alia Z . Effects of Amlodipine Besylate besylate on spermatogenesis in Sprague Dawley rats. Pak. Arm. F. Med. J. 2009: 4.
7. Cheng CY, Mruk DD. Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiol Rev. 2002;

82:825–74.
8. Lui WY, Mruk D, Lee WM, Cheng CY. Sertoli cell tight junction dynamics: their regulation during spermatogenesis. Biol Reprod. 2003; 68:1087–97.
9. Latif Rabia, Muhammad A, Tariq M. Spermatogenesis following discontinuation of calcium channel blocker Amlodipine Besylate in rats. J Ayub Med Coll Abbottabad. 2009; 21(1).
10. Gorczyńska-Fjälling E. The role of calcium in signal transduction processes in Sertoli cells. Reprod Biol. 2004; 4(3):219-41.
11. Almeida SA, Teofilo JM, Anselmo F, Brentegani LG, Lamano-Carvalho TL. Antireproductive effect of the calcium channel blocker Amlodipine Besylate in male rats. Exp. Toxicol. Pathol. 2000; 52:353-356.
12. Latif R, Lodhi GM, Hameed W, Aslam M. Steroidogenesis in Amlodipine Besylate treated purified Leydig cells. Toxicol Appl Pharmacol. 2012, 258(1):26-31.
13. Kumar N, Jain S, Gupta A, Tiwary AK. Spermicidal activity of sulfonylureas and meglitinide analogues: role of intrasperm Ca2+ elevation. J Pharm Pharmacol. 2008; 60(3):323-30.
14. McConnell EL, Basit AW, Murdan S. Measurements of rats and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J Pharm Pharmacol. 2008; 60(1):63 – 70.
15. Carafoli E, Santella L, Branca D, Brini M. Generation, control, and processing of cellular calcium signals. Crit Rev Biochem Mol Biol. 2001; 36: 107–260.
16. Bouschet T, Henley JM. Calcium as an extracellular signalling molecule: perspectives on the calcium sensing receptor in the brain. Comptes Rendus Biol. 2005; 328: 691–700.
17. Shimizu H, Nakagami H, Yasumasa N, Mariana OK, Kyutoku M, Koriyama H et al. Cilnidipine, but not amlodipine, ameliorates osteoporosis in ovariectomized hypertensive rats through inhibition of the N-type calcium channel. Hypertens Res. 2012; 35(1):77-81.