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AMLODIPINE BESYLATE IMPAIRS THE MORPHOLOGY OF BONE MARROW IN ADULT MALE WISTAR RATS

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ABSTRACT

Amlodipine is a long-acting calcium channel blocker used in the treatment of hypertension and angina. In adult man, the treatment regimen is 5 or 10 mg daily. This study evaluated the effects of prolonged oral administration of Amlodipine Besylate on the morphology of bone marrow in adult male Wistar rats. Sixteen rats (140 - 190 g) comprising of four groups were employed in the study. Rats of Control Group I received physiological saline orally while rats of Experimental Groups II - IV received oral administrations of 5, 10 and 15 mg/kg bodyweight of Amlodipine Besylate respectively for nine weeks. Histo-pathological examinations of the bone marrow showed normal cytoarchitecture of erythrocytes and leukocytes in rats of Control Group I. However, dose-dependent degeneration and lyses of erythrocytes and leukocytes were observed in amlodipine-treated rats. In conclusion, impaired morphology of the bone marrow were observed in amlodipine-treated adult male Wistar rats

Key words: Amlodipine Besylate, bone marrow, morphology, rats.

INTRODUCTION

Hypertension is a global health concern which affects all ethnic groups, 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 is unaware of their condition. (Tripathy, 2003; Nicholas et al., 2006; John and Arthur, 2011). Hypertension is usually treated with calcium channel blockers. (Tripathy, 2003). Amlodipine Besylate is a calcium ion antagonist which inhibits trans-membrane influx of calcium ion into cardiac and vascular smooth muscles (Tripathy, 2003; Nicholas et al., 2006; John and Arthur, 2011).

Calcium ions have ubiquitous presence in somatic and germ cells (Latif et al., 2008; Latif et al., 2009). Calcium ions are, therefore, of relevance in many biological processes such as bone formation, haemopoiesis, blood coagulation and co-factor for enzymes (Tripathy, 2003; John and Arthur, 2011) and signal transduction pathways where it is used as first and second messengers (Carafoli et al., 2001; Bouschet and Henley, 2005). Amlodipine Besylate as a calcium ion antagonist will possibly interfere with the processes involved in bone formation and hematopoiesis. It is, therefore, relevant to evaluate the possible effects of Amlodipine Besylate on the morphology of bone marrow. Following thorough literature review, we are not aware of any study that examined the effects of Amlodipine Besylate administration on the morphology of bone marrow of rats. This study evaluated the effects of prolonged oral administrations of Amlodipine Besylate on the morphology of bone marrow in adult male Wistar rats.

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MATERIALS AND METHODS

Ethical Approval

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

Sixteen adults male Wistar rats weighing 140 - 190 g obtained from the colony breed of the Animal House of the Department of Veterinary Physiology, University of Ibadan, Oyo State, Nigeria were employed in the study. Male rats were selected for the study to eliminate the possible effects of sex hormones such as Oestrogen, Follicle Stimulating Hormone and Luteinizing Hormone on the actions of Amlodipine Besylate in female rats. The male rats used for the study were housed in individual cages in a well ventilated and fumigated room with ambient temperature and good lighting. All rats were fed with standard pellet diet (Bendel Feeds Limited, 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 measured and computed daily. Furthermore, each rat was examined daily for possible behavioural and gross morphological or physical changes.

Administrations of Drugs

Rats of Control Group I (comprising of four rats) received physiological saline daily for nine weeks. In an adult 70 kg Man, the treatment regimen of Amlodipine Besylate for the treatment of hypertension or angina is 5 or 10 mg daily. 5 mg Amlodipine Besylate (Norvasc, Pfizer, New Zealand) was dissolved in 333 milliliters (ml) of Normal Saline effectively without any residue. Therefore, rats Experimental Groups II – IV (each comprising of four rats) received daily corresponding oral administration of 5, 10 and 15 mg/kg Bodyweight of Amlodipine Besylate respectively for 56 - 65 days. Oral administration of drugs was done with the use of a 5-ml syringe and a flexible feeding tube long enough to reach the stomach through the oesophagus.

The average weight of rats employed in the study was measured as 165 g. In an adult 70 kg Man, the treatment regimen of Amlodipine Besylate is 5 or 10 mg daily. Therefore, to determine the amounts of Amlodipine Besylate to be administered to each rat, the corresponding dosage (X mg) for a 165 g rat was calculated as follows: X mg = (165 g × 5 mg)/70,000 g = 0.012 mg of Amlodipine Besylate.

If 5 mg Amlodipine Besylate was dissolved in 333 ml of Normal Saline solution, the volume (X ml) of the Amlodipine Besylate/Normal Saline solution that would contain 0.014 mg of Amlodipine Besylate was determined as follows: X ml = (0.012 mg × 333 ml)/5 mg = 3.996 ml/5 = 0.80 ml (approximately 1 ml) of Amlodipine Besylate/Normal Saline solution.

The rats were divided into the following groups:

Group I: Control (4 ml saline, oral)

Group II: Treated (5 mg/kg b.w. or 1 ml Amlodipine Besylate/Normal Saline Solution, oral)

Group III: Treated (10 mg/kg b.w. or 2 ml Amlodipine Besylate/Normal Saline Solution, oral)

Group IV: Treated (15 mg/kg b.w. or 3 ml Amlodipine Besylate/Normal Saline Solution, oral)

Volumes of drugs solutions that were more than 2 ml were given twice daily to rats of Groups I and IV for eased ingestion. This was in consideration of the maximum 3.4 ml volume capacity of the stomach of adult rats (McConnell et al., 2008).

Histological Preparations of the Bone Marrow for Giemsa Staining Technique

At the end of experimental procedure, each rat was sacrificed by cervical dislocation. The lower limb was dissected; the femur was removed, fixed in 10% formal saline of at least five times

its volume. Bone marrow samples were aspirated from the femur. The microscope slides and spreader were cleaned thoroughly and ensured to be grease free. A drop of bone marrow aspirate was placed on each slide (at about 1cm from one edge of the slide) and the spreader was adjusted at an angle of about 45° to the slide. The spreader was drawn backward and forward with firm and smooth pressure. The smear was air-dried and the slide properly labeled.

Microscopic slides were placed on the staining rack, covered and fixed with methanol for five minutes. The slides were flooded with May-Grunwald solution and stained for fifteen (15) minutes. The stain was tipped off and slides flooded with Giemsa stain for 15 minutes. The stain was washed off under running water and the slides placed in the drying racks to air-dry. Slides were examined under the microscope using X 400 and oil immersion objective lens. (Lewis et al., 2003).

Histological Preparations of the Bone Marrow for Haematoxylin and Eosin Staining Techniques

The microscope slides and spreader were cleaned thoroughly and ensured to be grease free. A drop of bone marrow aspirate was placed on each slide (at about 1 cm from one edge of the slide) and the spreader was adjusted in such a way that it totally covered the slide and the bone marrow aspirate. The spreader was then drawn backward and forward with firm and smooth pressure. The smear was air-dried and the slide properly labeled. Microscopic slides were placed on the staining rack, stained with Haematoxylin, differentiated in 1% alcohol for twenty minutes and counter stained in eosin for three minutes. The stains were washed off in running water and the slides were placed in the drying rack to air-dry.

RESULTS

Histological evaluations showed that rats of Control Group I had normal cytoarchitecture of erythrocytes and leucocytes. However, dose-dependent degeneration and lyses of erythrocytes and leucocytes were observed in rats of Experimental Groups II – IV treated with 5, 10 and 15 mg/kg bodyweight of Amlodipine Besylate. (Figures 1a-d and 2a-d).

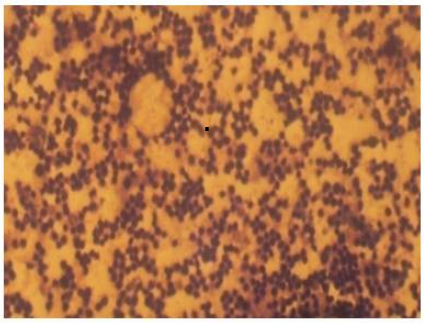

Figure 1a

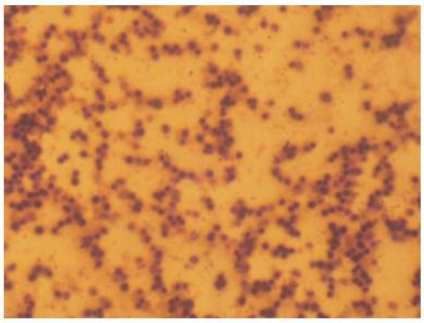

Figure 1b

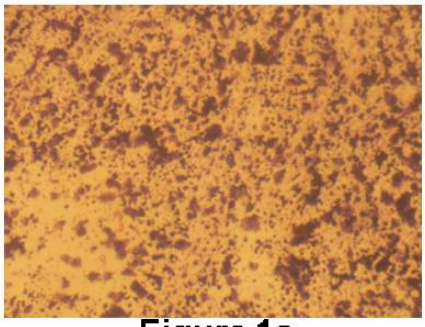

Figure 1c

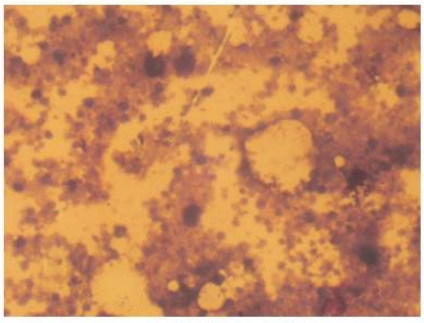

Figure 1d

Figure 1a: Photomicrograph sample of the bone marrow of rats of Group I which received physiological saline, showing multifoci and normal cytoarchitecture of erythrocytes and leucocytes. Figure 1b: Photomicrograph sample of the bone marrow of rats of Group II which received 5 mg/kg bodyweight of Amlodipine Besylate, showing mild degeneration and lyses of erythrocytes and leucocytes. Figure 1c: Photomicrograph sample of the bone marrow of rats of Group III which received 10 mg/kg bodyweight of Amlodipine Besylate, showing marked degeneration and lyses of erythrocytes and leucocytes. Figure 1d: Photomicrograph sample of the bone marrow of rats of Group IV which received 15 mg/kg bodyweight of Amlodipine Besylate, showing foci adipocytes, marked degeneration and lyses of erythrocytes and leucocytes. Giemsa X 400.

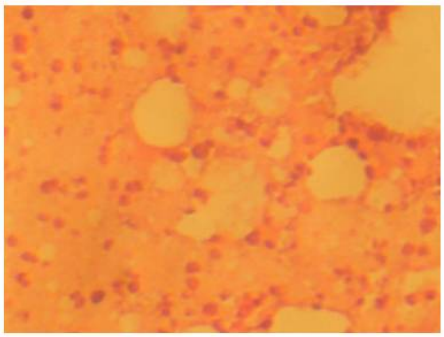

Figure 2a

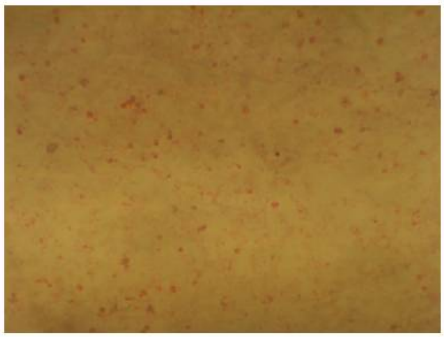

Figure 2b

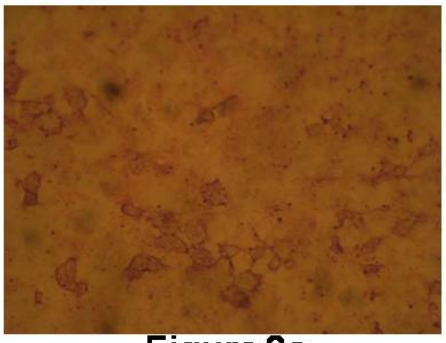

Figure 2c

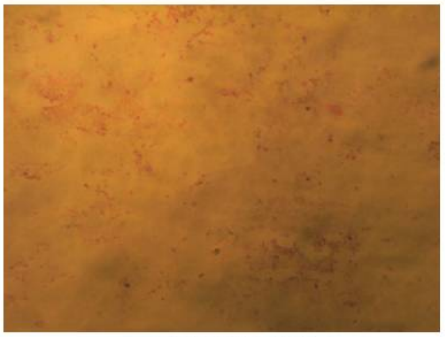

Figure 2d

Figure 2a: Photomicrograph sample of the bone marrow of rats of Group I which received physiological saline, showing normal nucleated erythrocytes and leucocytes. Figure 2b: Photomicrograph sample of the bone marrow of rats of Group II which received 5 mg/kg bodyweight of Amlodipine Besylate, showing degeneration and lyses of erythrocytes and leucocytes. Figure 2c: Photomicrograph sample of the bone marrow of rats of Group III which received 10 mg/kg bodyweight of Amlodipine Besylate, showing severe degeneration and lyses of erythrocytes and leucocytes. Figure 2d: Photomicrograph sample of the bone marrow of rats of Group IV which received 15 mg/kg bodyweight of Amlodipine Besylate, showing severe degeneration and lyses of erythrocytes and leucocytes. Haematoxylin and Eosin X 400.

DISCUSSION

Histological evaluation showed that rats of Control Group I had normal cytoarchitecture of erythrocytes and leucocytes. However, dose-dependent degeneration and lyses of erythrocytes and leucocytes were observed in rats of Experimental Groups II – IV treated with 5, 10 and 15 mg/kg bodyweight of Amlodipine Besylate. The observed anomalies of the morphology of bone marrow implied that Amlodipine Besylate as a calcium blocker negatively interacted with the functions of calcium ions as a major component of bones and regulator of haemopoiesis.

Literature review observed paucity of studies which evaluated the effects of Amlodipine Besylate on bone marrow of rats. However, the observations of this study are in agreement with earlier observations of adverse effects of Amlodipine Besylate on bone meatabolism in rats. Amlodipine Besylate administration was reported to have resulted in 20 - 30% decrease in bone volume fraction in the healing process of alveoli of rats following tooth extraction (Teofilo et al., 2001) and unlike Clinidipine did not prevent or ameliorate osteoporosis in ovariectomized hypertensive rats (Shimizu et al., 2012). 0.04 mg/rat/day Amlodipine Besylate resulted in slight delay in the chronology of the repair process, significant decrease of newly formed bone volume and alkaline phosphatase levels in the repairing process of surgical defect in the mandibular ramus of rats. (Moraes et al., 2011). Similarly, 0.3 mg/100 g bodyweight of Amlodipine Besylate administration resulted in increased expression of bone morphogenetic protein-2 (BMP-2) and decreased bone turnover rate in male wistar rats. (Gradosova et al., 2011).

The observations of this study are, however, in

disagreement with earlier studies which observed that 1 and 3 mg/kg bodyweight of Amlodipine Besylate resulted in significant increase in calcium and phosphorus concentrations in the femurs of ovariectomised rats with a beneficial effect on bone metabolism (Halici et al., 2008); and mitigated the effects of orchidectomy implying that it could prevent osteoporosis in rats. (Gradosova et al., 2012).

In conclusion, dose-dependent impaired morphology of the bone marrow was observed in adult wistar rats treated with 5, 10 and 15 mg/kg bodyweight of Amlodipine Besylate. This implied that Amlodipine Besylate should only be used based on medical prescription and should not be abused. Uses of Amlodipine Besylate against medical prescriptions to achieve assumed accelerated relief of hypertension by drug abusers could possibly result in adverse effects on the morphology of the bone marrow.

REFERENCES

1. Aguilar HN, Xiao S, Knoll AH, Yuan X. 2010. Physiological pathways and molecular mechanisms regulating uterine contractility. Human Reprod Update 16:725- 744.
2. Bouschet T, Henley JM. 2005. Calcium as an extracellular signalling molecule: perspectives on the calcium sensing receptor in the brain. Comptes Rendus Biol, 328:691- 700.
3. Carafoli E, Santella L, Branca D, Brini M. 2001. Generation, control, and processing of cellular calcium signals. Crit Rev Biochem, 36:107- 260.
4. Gradosova I, Zivna H, Svejkovska K, Palicka V, Tichy A, Zivny P. 2011. Effects of amlodipine on bone metabolism in male albino wistar rats. Acta Veterinaria Brno, 80:391- 396.
5. Gradosova I, Zivna H, Palicka V, Hubena S, Svejkovska K, Zivny P. 2012. Protective effect of amlodipine on rat bone tissue after orchidectomy. Pharmacol, 89:37- 43.
6. Halici Z, Borekci B, Ozdemir Y, Cadici E, Suleyman H. 2008. Protective effects of amslodipine and lacidipine on ovariectomy-induced bone loss in rats. European J Pharmacol, 579:241- 245.
7. John EH**,** Arthur CG. 2011. Guyton and Hall Textbook of Medical Physiology, 12th ed. USA: Saunders, Elsevier Limited.
8. Latif R, Ghulam ML, Muhammad A. 2008. Effects of Amlodipine Besylate on serum testosterone, testicular weight and gonado-somatic index in adult rats. J Ayub Med Coll Abbottabad 20: 8-10.
9. Latif R, Muhammad A, Mazhar HM, Idrees FB, Azhar M, Alia Z. 2009. Effects of Amlodipine Besylate on spermatogenesis in Sprague Dawley rats. Pakistan Armed Forces Med J, 59: 275 – 279.
10. Lewis SM, Bain BJ, Bates I. 2003. Dacie and Lewis Practical Heamatology. 7th ed. London: Blackwell Scientific Publication.
11. McConnell EL, Basit AW, Murdan S. 2008. Measurements of rats and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J Pharm Pharmacol, 60:63- 70.
12. Moraes R, Correa L, Luz J. 2011. Adverse effects of the Amlodipine on bone healing of the mandibular fracture: An experimental study in rats. Oral and Maxillofacial Surg, 15:93- 101.
13. Practice of Medicine. 20th ed. USA: Churchill Livingstone, Elsevier Limited.
14. Shimizu H, Nakagami H, Yasumasa N, Mariana OK, Kyutoku M, Koriyama H et al. 2012. Clinidipine, but not amlodipine, ameliorates osteoporosis in ovariectomized hypertensive rats through inhibition of the N-type calcium channel. Hypertension Res, 35:77- 81.
15. Teofilo JM, Brentegani LG, Carvalho LTL. 2001. A histometric study in rats of the effect of the calcium antagonist amlodipine on bone healing after tooth extraction. Arch Oral Biol, 46:375- 379.
16. Tripathy KD. 2003. Essentials of Medical Pharmacology. 5th ed. India: Jaypee Brothers Medical Publishers (P) Limited.