Research Article

Effect of Amlodipine (Calcium Channel Blocker) on Haematological and Biochemical Parameters in Male Wistar Rats

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Received: 18-08-2017; **Revised:** 21-10-2018; **Accepted:** 05-11-2018.

ABSTRACT

Twelve male rats (120 – 140 g) were divided into control (distilled water) and amlodipine-treated (0.7 mg/kg) groups (6 per group) for haematological and biochemical studies. The animals were orally treated on daily basis for 50 days. Red Blood Cell (RBC) count and Total White Blood Cell (TWBC) count were determined using haemocytometer. Activities of plasma Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), as well as levels of total protein, globulin, albumin, creatinine and Blood Urea Nitrogen (BUN) were determined by spectrophotometry. Data were analysed using descriptive statistics and ANOVA at p=0.05. Treatment of rats with amlodipine (0.7 mg/kg) produced insignificant ($p>0.05$) changes in PCV, Hb, RBC and TWBC values, but caused significant ($p<0.05$) reduction in MCH value relative to their respective controls. It can therefore be concluded that amlodipine probably has a little beneficial effect on haematological function in male rats.

Keywords: Amlodipine, Rats, Total white blood cell count, Red blood cell count, Total protein.

INTRODUCTION

Amlodipine is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina [1]. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Amlodipine does also act as functional inhibitor of acid sphingomyelinase [2].

Amlodipine is rapidly absorbed and is extensively metabolized in the liver while it shows linear dose-related pharmacokinetic characteristics and, at steady-state, there are relatively small fluctuations in plasma concentrations across a dosage interval[3]. Although structurally related to other dihydropyridine derivatives, amlodipine displays significantly different pharmacokinetic characteristics[4]. Amlodipine is a substrate of cytochrome P450 (CYP) 3A subfamily, specifically CYP3A4 [5-6]. In addition, amlodipine is also a P-glycoprotein (P-gp) substrate [7-8].

Amlodipine has been reported to have the potential to protect against acetaminophen-induced hepatotoxicity in rats[9]. Amlodipine has been reported to have anticonvulsant activity and also potentiated the anticonvulsant effect of phenytoin in MES model [10]. Amlodipine has been reported to potentiate the protective effect of zonisamide on pentylenetetrazol-induced kindling in mice [11]. Amlodipine has also been reported to reduce angiotensin II-Induced aortic aneurysms and atherosclerosis in hypercholesterolemic mice [12].

However, due to paucity of information from literature on the effect of amlodipine on haematological and biochemical parameters in male rats, this study therefore aims at investigating the effect of this antihypertensive agent on these aforementioned parameters in male rats.

MATERIALS AND METHODS

Experimental Animals

Adult male rats weighing between 120 g – 140 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Afe Babalola University Ethics Committee on guiding principles on care and use of animals.

Drug

Calcium channel blocker (amlodipine) tablets (TEVA UK, Ltd) were bought from Danax Pharmacy, Ibadan, Nigeria.

Amlodipine (10 mg) was dissolved in 10 ml of distilled water to give a concentration of 1.0 mg/ml.

The dosage of amlodipine used in this study was in accordance with that recommended by the manufacturer.

Experimental Design

Twelve male rats (120 – 140 g) were randomly divided into two groups, with each consisting of six animals. The two groups were subjected to the following oral treatments once a day for fifty (50) days:

Group I: received 0.5 mL/100 g of distilled water as control group.

Group II: received 0.7 mg/kg of amlodipine.

Collection of blood samples

Twenty four hours (day 51) after the last dosing of all the groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for hematological and plasma biochemical analyses. Before assays, the blood was centrifuged for 5 minutes using a bench top centrifuge (Centromix) and the plasma were used for the determination of the biochemical parameters.

Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the Improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to [13] using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method according to [14]. Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells [15]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to [13].

Determination of Plasma Biochemical Parameters

The total protein concentration was determined using the Biuret method [16] and the albumin concentration by the method of [17]. The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of [18]. The levels of creatinine, urea and alkaline phosphatase were determined using the method of [19]. All the above biochemical parameters were determined in the plasma using the Randox kits.

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p<0.05$.

RESULTS

The effect of amlodipine (0.7 mg/kg) on haematological and plasma biochemical parameters after treatment of rats for 50 days is shown in Tables 1 and 2 respectively.

Treatment of rats with amlodipine (0.7 mg/kg) produced no significant ($p>0.05$) changes in PCV, Hb, RBC TWBC, platelet, lymphocyte, neutrophil, monocyte, eosinophil, MCV and MCHC values relative to their respective controls.

Treatment of rats with amlodipine (0.7 mg/kg) caused significant ($p<0.05$) reduction in MCH value relative to the control.

Table 1: Effect of 50 days treatment with amlodipine on haematological parameters in male rats

Parameters	Control	Amlodipine (0.7 mg/kg)
PCV (%)	43.40 ± 0.52	40.60 ± 0.72
Hb (g/dL)	14.38 ± 0.34	13.30 ± 0.31
RBC ($\times10^6$/µL)	7.31 ± 0.32	6.82 ± 0.16
TWBC ($\times10^3$/µL)	3.70 ± 0.21	4.36 ± 0.32
Platelets ($\times10^5$/µL)	1.39 ± 0.05	1.24 ± 0.03
Lymphocytes (%)	71.00 ± 0.83	67.80 ± 0.93
Neutrophils (%)	25.40 ± 0.75	28.20 ± 0.86
Monocytes (%)	1.60 ±0.11	2.00 ±0.13
Eosinophils (%)	2.00 ±0.16	2.00 ± 0.17
MCV (FL)	59.36 ± 0.55	59.59 ± 0.54
MCHC (g/dL)	33.12 ± 0.41	32.73 ± 0.31
MCH (pg)	19.67 ± 0.34	19.51 ± 0.28*

(n=6, *p=0.05)

Treatment of rats with amlodipine (0.7 mg/kg) produced no significant ($p>0.05$) changes in total protein, albumin, globulin, ALT, AST, ALP, BUN and creatinine values relative to their respective controls.

Table 2: Effect of 50 days treatment with amlodipine on plasma biochemical parameters in male rats

Parameters	Control	Amlodipine (0.7 mg/kg)
Total Protein (g %)	6.80 ± 0.13	7.26 ± 0.21
Albumin (gm %)	2.66 ± 0.13	2.80 ± 0.15
Globulin (gm %)	4.14 ±0.18	4.46 ± 0.12
AST (µ/L)	42.20 ± 0.87	40.00 ± 0.76
ALT (µ/L)	29.60 ± 0.74	29.20 ± 0.86
ALP (IU/L)	110.00 ± 1.71	110.20 ± 1.34
BUN (mg/dL)	15.96 ± 0.28	16.26 ± 0.26
Creatinine (µmol/L)	0.74 ± 0.02	0.80 ± 0.01

(n=6, *p=0.05)

DISCUSSION

The result of the haematological study has shown that amlodipine caused no significant changes on the PCV, RBC and indices relating to it (Hb, MCV and MCHC). This could indicate that the drug does not have the potential to stimulate erythropoietin release from the kidneys which is the humoral regulator of RBC production [20]. It could also indicate that there were no changes in the oxygen carrying capacity of the blood and the amount of oxygen

delivered to the tissues since RBC and haemoglobin (Hb) are very important in transferring respiratory gases [21]. It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anaemic conditions while higher values are suggestive of polycythemia [22]; thus, the drug may not have the potential to induce anaemia or polycythemia. Also, the drug may not have adverse effects on the bone marrow, kidney and haemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism [23].

Amlodipine caused no significant change in TWBC value, which probably indicates that it has no effect on the ability of the body to defend against invading organisms[24]. Contrary result was reported by[25] in *Viscum album* extract treated rats.

Amlodipine caused no significant change in the platelet value, which probably indicates that it has no effect on the haemostatic function of the body. Contrary result was reported by [26] in *Fadogia agrestis* extract treated rats.

Amlodipine caused no significant change in lymphocyte value, which probably indicates that it has no effect on the acquired immune response of the body. Similar result was reported by [27] in isolated ergosterol treated rats.

Amlodipine caused no significant increase in the neutrophil count, which probably indicates it has no effect on the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (phagocytosis). Contrary result was reported [28] in *Dennettia tripetala* extract treated rats.

Amlodipine caused no significant change in the monocyte value, which probably indicates that it has no effect on the phagocytic function of the body [29]. Contrary result was reported by [30] in *Saccharomyces cerevisiae* extract fed hens.

Amlodipine caused no significant change in eosinophil value, which could indicate that it has no effect on the anti-allergic and anti-parasitic infectious responses of the body. Contrary result was reported by [31] in *Arctotis actotoides* extract treated rats and mice.

Amlodipine caused significant reduction in the MCH value, which probably indicates that it inhibits the induction of macrocytic anaemia, since increased MCV and MCH valves are known to be indicative of macrocytic anaemia [32]. Contrary result was reported by [33] in *Jatropha gossypifolia* extract treated rats. The insignificant change in the MCHC value caused by lisinopril probably indicates that it has no effect on induction of hereditary spherocytosis, since MCHC values are known to be elevated in hereditary spherocytosis. Similar result was reported by [33] in *Jatropha gossypifolia* extract treated rats.

The result of the plasma biochemical study has shown that treatment of rats with amlodipine caused insignificant change in total protein level. This might indicate that the drug has no effect on the buffering capacity of the blood as well as having no effect on colloid osmotic pressure, since plasma proteins have been reported to be responsible for 15% of buffering capacity of blood [24] and that osmotic pressure caused by the plasma proteins (called colloid osmotic pressure) tends to cause fluid movement by osmosis. Contrary result was reported by [34] in *Euphorbia heterophylla* extract treated rats.

Amlodipine caused no significant change in albumin level, which probably indicates that it has no effect on the plasma levels of metals, ions, fatty acids, amino acids, bilirubin and enzymes; since it has been reported that albumin serves as a carrier for metals, ions, fatty acids, amino acids, bilirubin, enzymes and drugs [24]. Contrary result was reported by [35] in *Enicostemma axillare* extract treated rats.

Amlodipine produced no significant change in globulin level, which probably indicates that it has no effect on both the natural and acquired immunity of the body against invading organisms, since it has been reported that globulins are principally responsible for the body's both natural and acquired immunity against invading organisms [29]. Similar result was reported by [36] in *Portulaca oleracea* extracts treated rats.

The insignificant change in the activity of AST caused by the drug could indicate it has no effect on induction of tissue necrosis, since it has been reported that elevation in the activity of AST can be associated with cell necrosis of many tissues, which allows leakage of large amounts of this enzyme into the blood [37]. Contrary result was reported by [38] in *Sida rhombifolia* extract treated mice and rats.

Amlodipine caused an insignificant change in the activity of ALT, which probably indicates that it has no effect on induction of hepatic damage, since it has been reported that ALT is present in the liver and other cells and is particularly useful in measuring hepatic necrosis, especially in small animals [39]. Contrary result was reported by [40] in *Moringa oleifera* extract treated rats.

Amlodipine caused no significant change in ALP level, this probably indicates the absence of cholestasis, since ALP has been reported to be a marker of cholestasis [41]. Similar result was reported by [33] in *Jatropha gossypifolia* extract treated rats.

Amlodipine induced insignificant changes in urea and creatinine levels, this probably indicates absence of nephrotoxicism (renal impairment), since high plasma levels of urea and creatinine are markers of kidney dysfunction. Contrary result was reported by [42] in *Passiflora edulis* extract treated rats.

CONCLUSION

In conclusion, this study has shown that amlodipine has a little beneficial effect on the haematological function in male rats. However, the effect of this antihypertensive agent on human haematological function and blood chemistry are unknown; nevertheless, considering these findings in animal model, it is recommended that patients should strictly comply with the dosage regimen as recommended by their physicians.

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Source of Support: Nil, **Conflict of Interest:** None.