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Full Length Research Article

Selected Antihypertensive Agents and their Fixed-Dose Combinations Effectively Ameliorate Trastuzumab-Mediated Cardiac Dysfunctions in Rats

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Summary: This study evaluates the therapeutic potentials of selected antihypertensive drugs [valsartan (VAL), amlodipine (ADP), lisinopril (LSP) and their fixed-dose combinations [(amlodipine + lisinopril) (ADP + LSP) and (valsartan + lisinopril) (VAL + LSP)] in ameliorating trastuzumab (*TZM*)-induced cardiovascular dysfunctions in experimental rats. In-bred female Wistar rats were randomly allotted into 10 groups of 6 rats per group. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) as well as electrocardiogram (ECG) of the treated rats were measured using non-invasive procedures on days 1 and 7 of the experiment, following which the treated rats were sacrificed under light inhaled diethyl ether and histopathological evaluation of all treated hearts was done. Results showed that repeated *TZM* treatment profoundly ($p<0.05$) raised SBP, DBP and MAP values from 115.0 ± 17.1 mmHg, 85.1 ± 15.1 mmHg and 94.7 ± 15.5 mmHg, respectively on day 1 to 127.7 ± 27.8 mmHg, 87.4 ± 27.3 mmHg and 100.5 ± 26.4 mmHg, respectively, on day 7. Oral pretreatments with VAL, ADP, LSP and their fixed-dose combinations profoundly ($p<0.05$) attenuated increases in the SBP, DBP and MAP values with the most significant attenuation mediated by the fixed-dose VAL + LSP combination at the SBP, DBP and MAP values of 103.8 ± 20.6 mmHg, 65.5 ± 18.8 mmHg, and 77.9 ± 18.7 mmHg, respectively. *TZM* treatment also profoundly ($p<0.05$) prolonged the QT and corrected QT intervals from 85.0 ± 11.5 ms and 161.6 ± 20.3 ms, respectively, on day 1 to 110.2 ± 21.5 ms and 226.5 ± 41.5 ms, respectively, on day 7. However, these QT and corrected QT interval prolongations were effectively and profoundly attenuated by oral pretreatments with VAL, ADP, LSP and their fixed-dose combinations. In addition, *TZM* cardiotoxicity was characterized by marked vascular and cardiomyocyte congestion and coronary artery microthrombi formation. However, these histopathological changes were reversed with oral pretreatments with ADP, LSP, VAL and fixed-dosed [(ADP + LSP) and (VAL + LSP)] combinations although fixed-dose VAL + LSP was associated with histopathological lesions of coronary arterial wall cartilaginous metaplasia. Overall, this study revealed the promising therapeutic potentials of VAL, ADP, LSP and their fixed-dose combinations as repurposed drugs for the prevention of *TZM*-mediated cardiac dysfunctions.

Keywords*: Valsartan, Amlodipine, Lisinopril, Trastuzumab cardiotoxicity, Blood Pressure parameters, ECG, Histopathology*

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INTRODUCTION

Cancer remains the second topmost major non-communicable disease worldwide, with estimated 18.1 million sufferers globally and an estimated 9.6 million deaths in 2018 resulting from complications of the disease (Siegel *et al.*, 2020). It is projected that by 2040, the global burden of cancer will grow to 27.5 million new cancer cases and 16.3 million cancer deaths as a result of population growth and ageing population (Rahib *et al.*, 2020). In Nigeria, an estimated yearly 10,000 cancer deaths and annual 250,000 new cases were reported (Akinde *et al.*, 2015). However, one of the novel targeted therapies in clinical cancer management involves the use of monoclonal antibody cytotoxics such as trastuzumab, pertuzumab, bevacizumab, margetuximab, atezolizumab, *etc.* (Costa and Czerniecki, 2020).

Trastuzumab (*TZM*) is a DNA-derived, human epidermal growth factor receptor type 2 (HER2) targeted recombinant monoclonal antibody directed against loco-regional and metastatic breast cancer (Piotrowsk *et al.*, 2011; Porta *et al.*, 2015, Fang *et al.*, 2020), gastric cancers (Lameire, 2015; Porta *et al.*, 2015), gastro-esophageal adenocarcinoma (Blackwell *et al.*, 2010; Poon *et al.*, 2013) and salivary duct carcinoma (Gibo *et al.*, 2019). *TZM* is reported to be clinically effective either as a monotherapy or in combination with other agents including the anthracyclines (ElZarrad *et al.*, 2013).

Undoubtedly, the clinical use of *TZM* has resulted in significant improvement in the prognosis of patients with advanced HER2-overtlyexpressing breast and gastric cancers but this use has reportedly been associated with cumulative but reversible off-target cancer therapy-related cardiac dysfunction (CTRCD) (Fang *et al.*, 2020; Brown *et al.*, 2020), either on acute or long-term use (Klein and Dybdal, 2003; Matos *et al.*, 2013; Hidalgo *et al.*, 2013; Mohan *et al.*, 2018). *TZM* is known to de-express myocardial genes, decrease left ventricular function and induce cardiomyocytes ultrastructural changes (ElZarrad *et al.*, 2013). *TZM* has also been reported to increase myocardial oxidative and nitrative stress and activates apoptotic pathways, resulting in profound elevations in the serum troponin-I and cardiac myosin light chain 1 (cMLC1) levels (ElZarrad *et al.*, 2013). Other reported *TZM*'s myocardial cytotoxicity include: disruption of signal transduction pathways, DNA disrepair, decreased angiogenesis, cell cycle disruption, and activation of antibody-dependent cellular cytotoxicity (Spector and Blackwell, 2009; Poon *et al.*, 2013, Lameire, 2015). *TZM* binds to the extracellular membrane domain of HER2 to inhibit proliferation and survival of HER2-dependent tumors after reversing the phenotype of HER2/neu expressing tumor cells (Drebin *et al.*, 1984; Mandaliya *et al.*, 2015). Clinically, acute *TZM* cardiotoxicity may manifest as myocardial dysfunction, ischemia, hypotension, hypertension, edema, prolonged QT-interval, arrhythmias and thromboembolism (Jones *et al.*, 2009; Alghafar *et al.*, 2020) while its long-term manifestations include progressive decline in left ventricular ejection fraction (LVEF) with subsequent left ventricle dysfunction, congestive cardiac failure, left bundle branch block (LBBB), and negative T-waves on ECG (Piotrowski *et al.*, 2012).

There are independent scientific reports on the therapeutic potentials of some of the known classes of antihypertensive agents in mitigating anthracycline- and trastuzumab-induced cardiotoxicities (Akolkar *et al.*, 2015; Rygiel, 2016; Wittayanukorn *et al.*, 2018; Blanter and Frishman, 2019; Brown *et al.*, 2020). These classes of antihypertensive agents include angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs) and beta(β)$_1$-adrenoceptor antagonists (Rygiel, 2016; Gujral *et al.*, 2018; Sharma *et al.*, 2018; Ma *et al.*, 2019; Guglin *et al.*, 2019). ACEIs and ARBs are notable antihypertensive and anti-cardiac failure agents that mediate their pharmacological action by regulating the renin-angiotensin-aldosterone system (RAAS)-dependent blood pressure homeostasis (Guo *et al.*, 2020). In heart failure patients, these agents have been reported to prevent and at times reverse left ventricular hypertrophy, hypertensive cardiomyopathy and heart failure, preserving cardiac function, and improving recovery prognosis (Gilbert, 1995; Gregory, 2001; Ruggenenti *et al.*, 2008; Goda and Masuyama, 2014; Messerli *et al.*, 2017). Similarly, other few small-scale randomized controlled trials (RCTs) have also reported the chemotherapeutic/chemopreventive potentials of ACEIs/ARBs may be in CTRCD (Pinter *et al.*, 2018; Blanter and Frishman, 2019). Unfortunately, results from these clinical studies have remained largely inconsistent (Boekhout *et al.*, 2016; Janbabai *et al.*, 2017; Gupta *et al.*, 2018; Guglin *et al.*, 2019). Thus, casting doubts on the efficacies of these therapeutic agents in *TZM* cardiotoxicity. Recently, the ameliorating effects of VAL, ADP, LSP and their fixed-dosed combinations in TZM-induced cardiotoxic rats that were mediated via reduced caspase-3 and caspase-9 expression and enhanced antioxidant mechanisms were reported (Olorundare *et al.*, 2021). This study, therefore, is a further study designed at evaluating the therapeutic potential of amlodipine (an angio-selective calcium channel blocker), lisinopril (a competitive angiotensin converting enzyme inhibitor), valsartan (angiotensin II receptor blocker) and their fixed-dose combinations in acute *TZM*-induced cardiotoxicity in Wistar rats outside their approved clinical use as antihypertensive and anti-cardiac failure regimen, thus, re-purposed. In doing this, effects of the oral pretreatments with these drugs and their fixed dose combinations on blood pressure parameters (systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure), electrocardiogram (P- wave duration, heart rate, QRS duration, QT segment, corrected QT segment, and R wave amplitude) and cardiac muscle histopathological endpoints were evaluated in *TZM*-induced cardiotoxicity.

MATERIALS AND METHODS

Drugs and Chemicals: Drugs used include amlodipine besylate (Pfizer Norvasc™ 5 mg, R-Pharm Germany GmbH, Heinrich-Mack-Str. 35, 89257 Illertissen, Germany), lisinopril dihydrate (Zestril™ 5mg, AstraZeneca Pharnaceutical Co., Ltd, Wuxi, Jiangsu, People's Republic of China), valsartan (Diovan® 160, Norvatis Pharma AG, Basel, Switzerland), xylazine, ketamine (Bayer, Germany). Chemicals such as hydrochloric acid (HCl), thiobarbituric acid (TBA), 1,2-dichloro-4-nitrobenzene, trichloroacetic acid (TCA), sodium hydroxide, xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade and were purchased from ThermoFisher Scientific, Cambridge, Massachusetts, U.S.A.

Experimental Animal Care: After an ethical approval (UERC Approval number: UERC/ASN/2020/2027) was obtained from the University of Ilorin Ethical Review Committee for Postgraduate Research, young adult female Wistar Albino rats (aged 8-12 weeks old and body weight: 170-190 g) were procured from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria. The procured rats were handled in accordance with international principles guiding the Use and Handling of Experimental Animals as provided by the

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). The rats were maintained on standard rat chow and potable water which were made available *ad libitum* and standard laboratory conditions (room temperature: 23-26 ^{o}C, relative humidity: 55 ± 5%, and controlled photoperiod of 12 hours light/12 hours dark periodicity).

Body weight Measurement: The body weights of rats were taken on days 1 and 7 of the experiment using a digital rodent weighing scale (®Virgo Electronic Compact Scale, New Delhi, India). The weight values obtained were expressed in grams (g).

Experimental Induction of TZM-induced cardiotoxicity and drug treatments of rats: *TZM*-induced cardiotoxicity was achieved via repeated intraperitoneal injections of 2.25 mg/kg of *TZM* as well as their oral pretreatments with VAL, ADP, LSP and their fixed-dosed combinations were as previously described by Olorundare *et al.* (2021).

Electrocardiography Measurement in treated rats: Electrocardiography was carried out using a 6-lead computer ECG machine using modified method of Adedapo *et al.* (2016). Briefly described, rats were placed on right lateral recumbency on an insulated board under light sedation with a proportional combination of ketamine (Ketavet®, 100 mg/ml, Pfizer, Berlin, Germany) and xylazine (Rompun® 2%, 20 mg/ml, Bayer, Leverkusen, Germany) (100 mg/kg ketamine + 5 mg/kg xylazine) administered intramuscularly to aid stabilization (Albrecht *et al.*, 2014). Skin fur was shaved to improve contact between the ECG pad and the skin as well as electrode gel was used to improve contact between the rat skin and ECG electrodes. The ECG's electrodes which were six in number were placed on both fore limbs, both hind limbs and chest of the treated rats. The electrodes were then connected to the ECG machine using color-coded cables while the ECG recording was done in a calm and quiet environment to avoid recording interference. The machine was calibrated and preset at 10 m/mV and 50 mm/s paper speed. From the standard lead-II tracings, ECG parameters such as heart rate, p-amplitude, PR-duration, R-amplitude, QRS complex, as well as QT/QTc parameters were evaluated. The corrected QT(QTc) was calculated using Bazett's formula:

$$QTc = \frac{\text{QT interval in seconds}}{\sqrt{\text{cardiac cycle in seconds}}} = \frac{QT}{\sqrt{RR}}$$

(Phan *et al.*, 2016)

Electrocardiography measurements were recorded on days 1 and 7 of the experiment.

Blood Pressure Measurement in treated rats: Blood pressure parameters such as systolic blood pressure, diastolic blood pressure and mean arterial pressure were measured in conscious but slight sedated rats by tail cuff plethysmography using CODA™ Non-invasive Computerized Blood Pressure Acquisition System (Kent Scientific, Torrington, Connecticut, USA). This non-invasive rat computerized blood pressure measurement was done as described by Gangwar *et al.* (2014) and Jayeola *et al.* (2020). For each rat, a total of nine readings were taken in the quiescent state after the animals had been well acclimatized to the procedure. Blood pressure parameter measurements were recorded on days 1 and 7 of the experiment. The Mean Arterial blood pressure (MAP) was calculated as:

Mean Arterial Blood Pressure (MAP) = {diastolic blood pressure + ($^{1}/_{3}$) × pulse pressure}, where pulse pressure (PP) = {systolic blood pressure (SBP) minus diastolic blood pressure (DBP)}

Harvesting and weighing selected vital organs: The hearts of treated rats were identified, freed of adjoining adventitia, dissected out *en bloc* and weighed on a digital weighing scale.

Histopathological evaluation of the heart tissues: The preparation of the heart tissues and their histopathologic evaluation were done using procedures earlier described by Olorundare *et al.* (2020).

Data Analysis: Data were presented as mean ± S.D. of six observations for the body weight parameters while data for the blood pressure and ECG measurements were expressed as mean ± S.E.M. of nine observations. Statistical analysis was done using One-way ANOVA followed by post-hoc Turkey's *post hoc* test, on GraphPad Prism Version 5. Statistical significance were considered at $p<0.05$, $p<0.001$, and $p<0.0001$.

Table 1.
Group treatment of rats

Groups	Treatments
Group I	Oral 10 ml/kg/day of sterile water. + 1 ml/kg/day of sterile water given *i.p.* for 7 days
Group II	Oral 5 mg/kg/day of valsartan in sterile water + 1 ml/kg/day of sterile water given *i.p.* for 7 days
Group III	Oral 0.25 mg/kg/day of amlodipine in sterile water + 1 ml/kg of sterile water given *i.p.* for 7 days
Group IV	Oral 0.035 mg/kg/day of lisinopril in sterile water + 1 ml/kg of sterile water given *i.p.* for 7 days
Group V	Oral 10 ml/kg/day of sterile water + 2.25 mg/kg/day of trastuzumab given *i.p.* for 7 days
Group VI	Oral 5 mg/kg/day of valsartan in sterile water + 2.25 mg/kg of trastuzumab given *i.p.* for 7 days
Group VII	Oral 0.25 mg/kg/day of amlodipine in sterile water + 2.25 mg/kg/day of trastuzumab given *i.p.* for 7 days
Group VIII	Oral 0.035 mg/kg/day of lisinopril in sterile water + 2.25 mg/kg/day of trastuzumab given *i.p.* for 7 days
Group IX	Oral 0.25 mg/kg/day of amlodipine + Oral 0.035 mg/kg/day of lisinopril *p.o.* + 2.25 mg/kg/day of trastuzumab given *i.p.* for 7 days
Group X	Oral 5 mg/kg/day of valsartan + Oral 0.035 mg/kg/day of lisinopril dissolved in sterile water + 2.25 mg/kg/day of trastuzumab given *i.p.* for 7 days

RESULTS

Effect of valsartan (VAL), amlodipine (ADP), lisinopril (LSP) and their fixed-dose combinations on body weights and body weight changes (% Δbwt.) *TZM*-treated rats: Table 2 shows the effect of repeated daily intraperitoneal injection with 2.25 mg/kg of *TZM* and their oral pretreatments with VAL, ADP, LSP and the fixed-dose combinations of ADP + LSP and VAL + LSP, respectively, on the average body weight and % Δbwt. on days 1 and 7. Repeated oral pretreatments with ADP and LSP to normal rats resulted in profound ($p<0.001$) reductions in % Δbwt. when compared to Groups I. Similarly, *i.p. TZM* treatment and oral pretreatments with ADP, LSP, VAL and their combinations caused similar significant ($p<0.001$) weight reduction in *TZM*-intoxicated rats when compared to untreated normal (Group I) rats (Table 2).

Table 2.
Effect of repeated oral treatment with valsartan, amlodipine, lisinopril and their fixed-dose combinations on the body weight and percentage body weight changes (% Δbwt.) of *TZM*-treated rats

Group	Day 1 body wt. (g)	Day 7 body wt. (g)	Δbwt.
I	208.60 ± 32.22	223.20 ± 35.12	06.95 ± 05.03
II	203.00 ± 17.43	216.70 ± 18.64	06.79 ± 03.83
III	200.60 ± 28.18	207.10 ± 30.61	03.25±02.76^{b-}
IV	206.10 ± 20.88	208.80 ± 18.82	02.12±01.91^{b-}
V	194.90 ± 11.56	195.30 ± 16.28	00.47±10.60^{b-}
VI	201.40 ± 16.36	204.40 ± 16.00	01.57±03.85^{b-}
VII	202.80 ± 15.36	209.30 ± 19.05	03.13±02.99^{b-}
VIII	204.60 ± 13.59	210.80 ± 06.87	02.98±03.70^{b-}
IX	207.00 ± 10.24	201.80 ± 13.34	01.80±03.17^{b-}
X	194.80 ± 20.82	198.90 ± 24.05	02.08±03.63^{b-}

$^{b-}$ represents a significant decrease at $p<0.001$ when compared to untreated normal control (Group I) and valsartan-treated rats (Group II).
Group I - 10 ml/kg/day sterile water given p.o. + 1 ml/kg/day sterile water given i.p.; Group II - 5 mg/kg/day valsartan given p.o. + 1 ml/kg/day sterile water given i.p. ; Group III - 0.25 mg/kg/day amlodipine given p.o. + 1 ml/kg sterile water given i.p. ; Group IV - 0.035 mg/kg/day lisinopril given p.o. + 1 ml/kg sterile water given i.p.; Group V - 10 ml/kg/day sterile water given p.o. + 2.25 mg/kg/day TZM given i.p.; Group VI - 5 mg/kg/day valsartan given p.o. + 2.25 mg/kg TZM given i.p.; Group VII - 0.25 mg/kg/day amlodipine given p.o. + 2.25 mg/kg/day TZM given i.p; Group VIII - 0.035 mg/kg/day lisinopril given p.o. + 2.25 mg/kg/day TZM given i.p.; Group IX - 0.25 mg/kg/day amlodipine + 0.035 mg/kg/day lisinopril given p.o. + 2.25 mg/kg/day TZM given i.p.; Group X - 5 mg/kg/day valsartan + 0.035 mg/kg/day lisinopril given p.o. + 2.25 mg/kg/day TZM given i.p

Effect of valsartan (VAL), amlodipine (ADP), lisinopril (LSP) and their fixed-dose combinations on SBP, DBP and MAP of *TZM*-intoxicated rats: The baseline blood pressure parameters (SBP, DBP and MAP) of treated rats on day 1 of treatment were not significantly different from one group to another (Table 3). With repeated *i.p. TZM* treatment, there were profound ($p<0.05$) increases in the SBP, DBP and MAP from 115.0±17.1 mmHg, 85.1±15.1 mmHg and 94.7±15.5 mmHg, respectively, on day 1 (Table 3) to 127.7±27.8 mmHg, 87.4±27.3 mmHg and 100.5±26.4 mmHg, respectively, on day 7, respectively in the untreated *TZM* intoxicated (Group V) rats on day 7 of treatment (Table 4). However, with repeated oral pre-treatments with VAL, ADP, LSP and their fixed-dose combinations, increases in these parameters were significantly ($p<0.05$) attenuated with the most significant attenuation offered by the fixed-dose VAL + LSP combination (Table 4).

Effect of valsartan (VAL), amlodipine (ADP), lisinopril (LSP) and their fixed-dose combinations on ECG parameters on days 1 and 7 of treatment: Table 5 shows the baseline ECG parameters (P wave duration, HR, QRS duration, QT interval, corrected QT interval and R-wave interval) of treated on day 1. Following repeated *i.p. TZM* injection to treated rats for 7 days, the QT intervals and corrected QT intervals were significantly ($p<0.05$) increased from 85.0 ± 11.5 ms and 161.6 ± 20.3 ms, respectively, on day 1 (Table 5), to 110.2 ± 21.5 ms and 226.5 ± 41.5 ms, respectively, in untreated *TZM* intoxicated (Group V) rats (Table 6 and Figure 1E) when compared to 89.5 ± 16.0 ms and 167.5 ± 48.3 ms, respectively, in untreated control (Group I) values (Table 6 and Figure 1A). However, with repeated oral pretreatments with VAL, ADP, LSP and their fixed-dose combinations, prolongation in the QT and corrected QT intervals were significantly ($p<0.05$) attenuated (Table 6, Figures 1B-1D, 1F-1I) with the most profound attenuation ($p<0.05$ and $p<0.001$) offered by VAL+LSP fixed-dose combination (Table 6, Figures 1J).

Table 3.
Baseline SBP, DBP and MAP before *TZM*-intoxication and oral pretreatments with valsartan, amlodipine, lisinopril and their fixed-dose combinations in allotted groups of Wistar rats on day 1

Treatment Groups	Systolic Blood Pressure (SBP) (mmHg)	Diastolic Blood Pressure (DBP) (mmHg)	Mean Arterial Pressure (MAP) (mmHg)
I	108.3 ± 21.1	73.9 ± 18.5	85.0 ± 18.0
II	112.0 ± 22.0	76.4 ± 19.6	88.0 ± 19.2
III	106.8 ± 26.7	76.1 ± 29.8	86.0 ± 28.2
IV	107.9 ± 20.6	74.2 ± 18.6	85.1 ± 18.6
V	115.0 ± 17.1	85.1 ± 15.1	94.7 ± 15.5
VI	116.7 ± 23.8	82.6 ± 23.0	93.7 ± 22.7
VII	100.9 ± 19.7	67.1 ± 16.0	76.3 ± 17.4
VIII	102.3 ± 15.4	71.8 ± 16.9	81.7 ± 15.4
IX	112.2 ± 23.3	79.9 ± 22.5	90.3 ± 22.0
X	115.3 ± 29.1	79.4 ± 25.3	90.9 ± 25.8

Table 4.
Effect of oral pretreatment of valsartan, amlodipine, lisinopril and their oral fixed-dose combinations in on SBP, DBP and MAP in *TZM*-intoxicated rats on day 7

Treatment Groups	Systolic Blood Pressure (SBP) (mmHg)	Diastolic Blood Pressure (DBP) (mmHg)	Mean Arterial Pressure (MAP) (mmHg)
I	109.1 ± 22.7	69.6 ± 20.5	82.5 ± 20.7
II	122.3 ± 35.6	85.5 ± 30.7	97.5 ± 31.9
III	115.5 ± 19.0*$^{-}$	83.7 ± 15.0	93.9 ± 15.7
IV	107.6 ± 19.6*$^{-}$	72.0 ± 21.7*$^{-}$	83.6±20.3*$^{-}$
V	127.7 ± 27.8*	87.4 ± 27.3*	100.5 ± 26.4*
VI	108.6 ± 27.9*$^{-}$	69.2 ± 18.3*$^{-}$	82.1 ± 20.1*$^{-}$
VII	115.8 ± 30.0*$^{-}$	74.9 ± 26.4*$^{-}$	88.2 ± 26.9*$^{-}$
VIII	137.7±26.9*	92.3 ± 24.5*	107.0 ± 23.7*
IX	113.6 ± 22.7*$^{-}$	69.1 ± 18.7*$^{-}$	83.6 ± 18.7*$^{-}$
X	103.8 ± 20.6	65.5 ± 18.8	77.9 ± 18.7

** represents a significant increase at $p<0.05$ when compared to untreated normal (Group I) values while*
**$^{-}$ represents a significant decrease at $p<0.05$ when compared to untreated TZM-intoxicated (Group V) values*

Table 5.
Baseline ECG parameters before *TZM*-intoxication and oral pretreatments with valsartan, amlodipine, lisinopril and their fixed-dose combinations in allotted groups of Wistar rats on day 1.

Treatment Groups	P-duration (ms)	HR (/min)	QRS duration (ms)	QT segment (ms)	QT segment (corrected) (ms)	R amplitude (mV)
I	16.8±2.8	221.0±34.7	16.6±2.1	94.6±14.4	181.2±33.0	0.47±0.08
II	22.5±2.9	225.0±42.3	16.2±1.7	76.8±18.0	147.3±33.7	0.42±0.09
III	21.2±3.3	240.±27.5	13.8±1.1	63.2±6.9	125.4±12.6	0.31±0.06
IV	18.0±3.5	215.8±22.3	13.7±2.2	83.8±8.4	158.7±23.5	0.55±0.15
V	17.6±4.0	220.2±32.6	15.4±3.8	85.0±11.5	161.6±20.3	0.52±0.20
VI	21.0±5.1	216.4±12.3	14.4±2.7	96.8±11.3	182.8±19.1	0.38±0.12
VII	25.6±3.6	247.8±26.1	15.0±0.7	89.4±13.4	179.8±17.8	0.48±0.11
VIII	21.5±3.8	205.7±25.9	15.2±2.6	97.7±11.7	179.0±14.8	0.51±0.18
IX	21.2±2.9	221.7±15.9	14.7±0.8	100.2±17.4	191.5 ±31.9	0.49±0.16
X	20.4±3.0	238.0±28.4	13.8±1.3	84.0±14.3	165.8±24.3	0.44±0.08

Table 6.
Effect of valsartan, amlodipine, lisinopril and their fixed-dose combinations on the ECG parameters in *TZM*-intoxicated rats on day 7

Treatment Groups	P-duration (ms)	HR (/min)	QRS duration (ms)	QT segment (ms)	QT segment (corrected) (ms)	R amplitude (mV)
I	18.0±3.0	259.5±11.8	14.2±2.4	89.5±16.0	167.5±48.3^{**-}	0.39±0.13
II	18.2±6.3	217.5±29.0^{*-}	13.3±2.6	70.5±23.1^{*-}	150.8±27.9^{*-}	0.42±0.14
III	17.5±3.7	243.7±12.6	14.0±1.5	91.2±15.3^{*-}	190.0±19.1	0.47±0.04
IV	20.0±3.4	257.3±17.5	15.2±3.7	93.0±15.78^{*-}	191.8±29.5^{*-}	0.50±0.08
V	20.0±2.5	255.7±12.4	13.5±3.0	110.2±21.5*	226.5±41.5*	0.47±0.08
VI	21.0±2.5	250.4± 15.9	14.6±2.2	75.6±4.0^{*-}	153.8±8.3^{*-}	0.40±0.16
VII	18.8±6.1	221.6±10.6^{*-}	14.6±0.5	82.0±13.2^{*-}	157.6±28.4^{*-}	0.58±0.12
VIII	16.8±4.0	219.6±37.7^{*-}	16.0±1.2	65.8±11.8^{**-}	129.0±25.9^{***-}	0.46±0.14
IX	15.3±6.1	252.0±28.6	16.3±0.6	79.3±8.1^{*-}	161.0 ±7.8^{*-}	0.39±0.07
X	16.7±2.3	222.3±13.5^{*-}	14.3±1.0	72.8±9.2^{*-}	139.5±19.0^{**-}	0.41±0.12

* *represents a significant increase at p<0.05 when compared to untreated normal control (Group I) values while* $^{*-}$ *,* $^{**-}$ *and* $^{***-}$ *represent significant decrease at p<0.05, p<0.001 and p<0.0001, respectively, when compared to untreated TZM-intoxicated (Group V) values.*

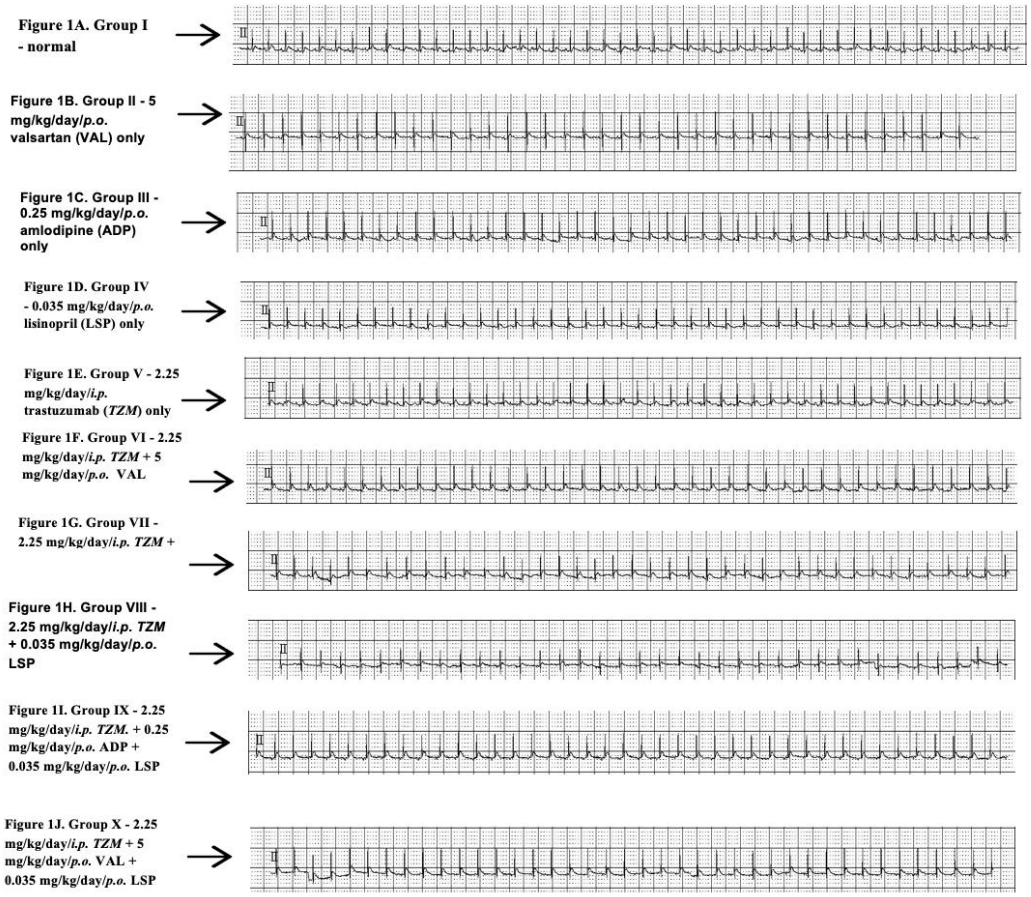

Figure 1.
ECG tracings showing effects of amlodipine (ADP), valsartan (VAL), lisinopril (LSP) and their fixed-dose combinations on the P wave duration, HR, QRS duration, QT interval, corrected QT interval and R-wave interval in *TZM*-intoxicated rats. *TZM* intoxication was characterized by prolonged QT intervals (Figure 1E) which were profoundly attenuated by oral pretreatments with VAL, ADP and LSP (Figures 1B-1D, respectively) and their fixed dose combinations (Figures 1F-J) with the ECG parameters returning to about normal (Figure 1A).

DISCUSSION

HER-2 targeted agents such as trastuzumab, pertuzumab, lapatinib and other congeners, remain the gold standard treatment strategy for both early and metastatic HER2-positive breast cancers, which are marked by the overexpressed HER2 genes (Sendur *et al.*, 2013; Perez *et al.*, 2019). The antibody targeted therapy, *TZM*, is notoriously reputed for its non-dose dependent, cumulative but reversible off-target cancer therapy-related cardiac dysfunction, especially on prolonged use. Trastuzumab-induced cardiotoxicity is believed to be the result of attenuated cardiac HER2-mediated signaling, ultimately resulting in decreased cardiomyocyte functionality with HER2 functioning as a compensatory mechanism against anthracycline-related cardiac stress. This is believed to be mediated via two processes namely: an increased activation of HER2-HER4-mediated cardiomyocyte survival pathways and cardiac dysfunction through inhibition of the HER2-HER4-mediated signaling (Perik *et al.*, 2007). Thus, *TZM* causes type II chemotherapy related cardiotoxicity mediated partly through ErbB2 pathway, which is dose independent, largely reversible and does not produce ultrastructural changes on histological examination (Tsang and Moe, 2007; Hamed *et al.*, 2016).

Previous studies have reported the protective role that some classes of antihypertensive agents play in ameliorating *TZM*- and anthracycline-induced cardiotoxicity (Rygiel, 2016; Brown *et al.*, 2020). These classes include cardioselective β_1-blockers (e.g. bisoprolol, carvedilol, metoprolol, *etc*) (Gulati *et al.*, 2016; Pituskin *et al.*, 2017; Gujral *et al.*, 2018; Guglin *et al.*, 2019; Brown *et al.*, 2020), calcium channel blockers (e.g. amlodipine), angiotensin converting enzyme inhibitors (e.g. lisinopril, enalapril, *etc*) (Vaduganathan *et al.*, 2019; Guglin *et al.*, 2019; Brown *et al.*, 2020) and angiotensin receptor blockers (e.g. losartan, valsartan, telmisartan, candesartan, *etc*) (Gulati *et al.*, 2016; Gujral *et al.*, 2018; Boekhout *et al.*, 2016), although there have been conflicting results with differing magnitudes of therapeutic benefits of these antihypertensive classes of drugs (Gujral *et al.*, 2018). Similarly, ACEIs and β-blockers have been reported to be effective at improving LVEF in non-ischemic cardiomyopathy, including chemotherapy-mediated LV dysfunction, especially with prolonged trastuzumab use (Vejpongsa and Yeh, 2014; Leong *et al.*, 2019). In view of these drawbacks, the present study was aimed at evaluating the therapeutic potential of amlodipine, lisinopril, valsartan, individually as well as their fixed-dose combinations in ameliorating *TZM*-associated cardiotoxicity in experimental rats using reliable cardiovascular parameters like blood pressure parameters (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate), ECG parameters as well as histopathological endpoints.

TZM is reported to cause profound acute coronary syndrome, left ventricular systolic dysfunction (LVSD) (manifesting as LVEF reductions) (Hildago *et al.*, 2013; Florido *et al.*, 2017, Tahir *et al.*, 2019; Chen *et al.*, 2020), cardiac failure (Florido *et al.*, 2017) systemic hypertension (Hildago *et al.*, 2013; Chen *et al.*, 2020) and most recently pulmonary arterial hypertension (PAH) following its prolonged use (Kowalczyk *et al.*, 2017; Umoru *et al.*, 2020) although other school of thought believes that the pre-existing hypertension, diabetes mellitus and obesity/hyperlipidemia as well as advanced age are known major risk factors for *TZM* cardiotoxicity (Jawa *et al.*, 2016; Eiger *et al.*, 2020).

Electrocardiogram (ECG) remains effective screening tool and gold standard for the diagnosis of LVSD (Olesen *et al.*, 2015; Boonman-de Winter *et al.*, 2015) and may help determine the underlying cause of LVSD (Khunti *et al.*, 2004; Davenport *et al*., 2006). However, LVSD is often characterized by five major EGC correlates which are abnormal Q-waves, atrial fibrillation, ventricular racing, left bundle branch block and prolonged QRS duration (Olesen and Andersen, 2016). Similarly, ECG manifestations of *TZM* cardiotoxicity include abnormal P wave, anterolateral T wave inversions (otherwise known as negative T wave), and prolonged QT segment (indicating bundle branch block) (Piotrowski *et al.*, 2012; Hildago *et al.*, 2013). Therefore, the fact that in this study, repeated *TZM* treatment was associated with elevated blood pressure parameters such as SBP, DBP and MAP suggested that *TZM* cardiotoxicity was fully established. Similarly, *TZM* intoxication was associated with prolonged QT segment suggesting the establishment of bundle branch block. Hence, this result is in complete agreement with earlier reports that prolonged *TZM* use could cause bundle branch block (Piotrowski *et al.*, 2012; Tahir *et al.*, 2019). The fact that oral pretreatments with VAL, ADP, LSP and their fixed-dose combination effectively attenuated increases in the measured blood pressure parameters and QT duration are strong indications of the therapeutic potential of these drugs in ameliorating *TZM*-mediated systemic hypertension. Although, the exact mechanism(s) of ameliorating *TZM*-mediated cardiotoxicity were not investigated in this study but could related to heart remodeling, injury reperfusion and cardiomyocytes anti-apoptosis activities of these drugs, especially ACEI and ARBs, which have previously been reported (Iqbal *et al*., 2008; Zablocki and Sadoshima 2013; Akolkar *et al.*, 2015). On molecular basis, angiotensin II is an effective downregulator of the actions of the NRG-1/ErbB system (Lemmens *et al.*, 2006; Vermeulen *et al.*, 2017), and strongly suggesting that the beneficial role of ACE inhibition may be related to this effect (Munster *et al.*, 2019). Thus, lisinopril, could be mediating its cardioprotective mechanism via the heart remodeling pathway, most likely through reduced caspase-3 and caspase-9 production and increased antioxidant mechanisms, which were the mechanisms through which VAL, ADP, LSP and their fixed-dose combinations mediated their mitigating effect in *TZM*-induced cardiotoxicity (Olorundare *et al.*, 2021).

TZM is known to cause related cardiac dysfunction without corresponding histoarchitectural distortion of the myocytes (Jones *et al.*, 2009) although *TZM* was recently reported to induce severe vascular congestion and associated microthrombi formation without attendant significant alterations in the myocyte histoarchitecture (Olorundare *et al.*, 2020) in treated experimental rats which the present study is in tandem with. However, the fact that these histopathological changes were profoundly improved by ADP, VAL and fixed-dose (ADP + VAL) combination

pretreatments, strongly suggest the cardioprotective potential of these drugs. Another important finding of this study is the histological finding of coronary artery cartilaginous metaplasia which was a prominent cardiac histopathological lesion found in rat hearts on LSP- and fixed-dose VAL + LSP combination pretreatments. Vascular cartilaginous/osseous metaplasia, which classically features the presence of arterial chondrocytes expressing type II collagen, is known to be part of the progression of mineralization or atherosclerotic lesion (Qiao *et al.*, 1995; Wallin *et al.*, 2001; Fitzpatrick *et al.*, 2003; Nguyen *et al.*, 2012). It also provides evidence of cardiac extracellular matrix remodeling for post-infarcted heart and may constitute a supplemental factor for heart failure when it calcifies (Manole *et al.*, 2019; Carreon *et al.*, 2020). Thus, cartilaginous metaplasia is seen as a potential pathway for artery wall calcification associated with the atherosclerotic plaque (Qiao *et al.*, 1995). Thus, the marked presence of coronary artery cartilaginous metaplasia as seen in the fixed-dose valsartan-lisinopril (VAL + LSP) combination-pretreated heart is suggestive of the either vascular remodeling of the *TZM*-mediated endothelial injury or coronary artery atheromatous plaque formation. However, the former appears to be more likely as the histological finding of coronary artery recanalization was observed with the drug combination pretreatment.

Overall, findings of this study highlight the promising therapeutic potentials of the antihypertensives – amlodipine, lisinopril, valsartan and their fixed-dose combinations as repurposed therapeutics in the management of *TZM*-induced cardiac dysfunctions.

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