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Ocimum Gratissimum Linn. Leaf Extract and Fractions Pre-Treatments are not Associated With Deleterious Electrocardiogram Changes in Trastuzumab-Intoxicated Wistar Rats

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Abstract

Trastuzumab (*TZM*) treatment is known to be associated with arrhythmogenic potential which primarily is the basis for its cardiotoxicity. The purpose of this study was to investigate the acute influence of oral pretreatments with 100 mg/kg/day of *Ocimun gratissimum* ethanolic leaf extract (*OG*) and its fractions (petroleum ether, *PEOG*; ethyl acetate, *EAOG*; and ethanolic extract, *EOG*) as well as valsartan-lisinopril fixed dose combination (VAL-LSP) on electrocardiogram (ECG) of Wistar rats intaperitoneally treated with 2.25 mg/kg/day *TZM* for 7 days. Young adult male Wistar rats were randomly allotted into 12 groups of 6 rats per group. The rats were subjected to electrocardiograms (ECG) measurement using non-invasive procedures on days 1 and 7 of the experiment. Results showed that oral pretreatment with *OG* and its fractions (except *EOG*) as well as VAL-LSP fixed dose combination did not cause any remarkable changes in the ECG patterns of *TZM*-treated rats indicating that their relative oral safety in *TZM* chemotherapy. On the other hand, *EOG* pretreatment caused significant shortening of the OT/QTc interval in the *TZM*-treated rats highlighting the arrhthymogenic potential of this fraction. Overall, the study highlighted the arrhythmogenic potential of *EOG* in *TZM* chemotherapy while *OG* and its other solvent fractions as well as VAL-LSP could be considered relatively safe for use as adjuvants in *TZM* chemotherapy.

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1. Introduction

Trastuzumab, popularly known and sold as Herceptin®, is a purified recombinant DNA-based humanized monoclonal antibody that targets the human epidermal growth factor receptor 2 (HER2) approved by the Food and Drug Administration (FDA) for the clinical management of HER2-positive metastatic breast cancer [1-2] and other HER2-expressing solid tumors such as gastro-esophageal adenocarcinoma, gastric cancer and salivary gland tumors [3-4]. *TZM* induces its cytotoxic action via multiple and complex mechanisms that include

HER2 internalization and degradation, antibody-dependent cellular cytotoxicity, and MAPK and PI3K/Akt interference [5-6]. However, despite being a gold standard in breast cancer treatment, *TZM* is notorious for causing off-target cardiotoxicity and treatment resistance although other important cardiac complications of *TZM* use previously reported include asymptomatic left ventricle dysfunction or symptomatic heart failure [7]. *TZM*-induced cardiotoxicity is postulated to be mediated via multiple mechanisms that include HER2 signaling dysregulation, cardiomyocyte autophagy suppression, and cardiomyocyte reactive oxygen species (ROS) accumulation [8-9] with all these culminating in accelerated cardiomyocyte apoptosis [8, 10]. *TZM*, like doxorubicin (an anthracycline cytotoxic agent), also binds to the DNA topoisomerase IIB (TOP2B) protein to cause DNA breakage, activation of DNA damage response pathway, and cardiomyocytes apoptosis [10]. There have been extensive reports and documentations on the *TZM*-associated left ventricular (LV) dysfunction (LVD) and heart failure (LVHF) but emerging and accumulating reports showed that *TZM* treatment equally often induces subtle but significant deleterious and fatal effects on the right ventricle (RV) structure and function that are mostly unrecognized [11-12]. Indeed, RV heart failure (RVHF) has been documented to co-exist with LVFH [13] and may be irreversible even after the discontinuation of chemotherapy unlike LVHF [14]. Other studies have demonstrated that TZM blocks the HER2 receptors in the cardiomyocytes rendering both the right and left ventricles vulnerable to type II or reversible cardiotoxicity [15-16]. However, the thinner structure of the RV with lesser myofibrils may make it more vulnerable to the *TZM* toxicity [17]. There are reports that *TZM* treatment is associated ECG changes that include new negative T-waves in ECG, ventricular bigeminal rhythm, changes in the T-wave or ST-segment (depression or elevation), unspecified arrhythmias, sinus bradycardia, asymptomatic left and right bundle branch blocks,atrial fibrillation, and non-sustained ventricular tachycardia [18-20]. Thus, the need for the periodic electrocardiographic assessment in patients on *TZM* chemotherapy becomes imperative [20]. *Ocimum gratissimum* Linn. (Lamiaceae) is an aromatic perennial medicinal and ornamental plant that is commonly known as clove basil, African basil and wild basil [21]. It is widely used as food seasoning and condiment in the West African and Asian cuisines [22-23]. Its local names include "Scent leaves" (Nigeria), "Nunu Bush" (Jamaica), "Fobazen" (Haiti), "Mujaaja" (Uganda) and "Maduruthala" (Sri Lanka) [24]. *Ocimum gratissimum* Linn. known to elicit different biological activities that include anti-stress [25], antidiarrheal [26-27], anti-helminthic, anti-inflammatory, antipyretic [28-30], antioxidant and free radical scavenging [29, 31], antimutagenic and anticancer [32], anti-ulcerative and gastroprotective, antibacterial [24], fungicidal [33], hepatoprotective [34], antiapoptotic [35]. Its other biological activities include male anti-fertility and aphrosidiac [36-37], and sedative activities [38-39]. Polyphenol-rich fraction of Ocimum gratissimum Linn. leaves have reportedly protected against cyclophosphamide-induced nephrotoxicity [40], gentamicin-induced hepatotoxicity [41] as well as lead acetate-induced cerebellar dysfunction [42] in rats. Other studies have also reported the safety and male fertility-enhancing effect of both the ethyl acetate and n-butanol fractions of *Ocimum gratissimum* Linn. leaf extract in Wistar rats [37]. While *Ocimum gratissimum* has been reported to possess antihyperlipidemic, anti-ischemic, and antihypertensive activities [43], a bioactive compound isolated from the plant leaves. gratissinol, is documented to be responsible for its cardioprotective activities [44]. In our quest to provide effective and affordable therapeutic option(s) for treating *TZM*-induced cardiotoxicity, this study was designed at conducting electrocardiographic safety assessment of the oral pretreatments with *Ocimum gratissimum* Linn. leaf extract and its different solvent fraction in acute *TZM*-intoxicated rats.

2. Materials and Methods

2.1. Drugs and chemicals

The drugs and chemicals used for this study are as reported by Olorundare *et al.* [4, 45].

2.2. Collection of plant materials

10 kg of the fresh whole plant of *Ocimum gratissimum* L. was collected from the thick forest of Moro Local Government Area of Kwara State, Nigeria in the month of June 2020. The plant was botanically identified and authenticated by Mr. Bolu Ajayi, a Senior Herbarium Curator at the University of Ilorin Herbarium. A voucher specimen with reference number UILH/001/984/2002 was prepared and deposited in the herbarium. The rest

of the collected plant samples were gently rinsed under flowing tap water, de-stalked and air-dried under shade in the laboratory at room temperature of 28-32℃ for 3 weeks. The completely air-dried plant leaves were pulverized using Laboratory Hammer mill and stored in air- and water-tight containers and kept at room temperature in the laboratory until needed for extraction.

2.3. Extraction processes and calculation of %yield

Pulverized sample of *Ocimum gratissimum* Linn. dried leaves (2.6 kg) was soaked in 5.2 L of 99.7% ethanol for 72 hours after which it was continuously stirred for 1 hour before it was filtered using 180 mm of filter paper. The filtrate was then concentrated at 40˚C to complete dryness using rotary evaporator. The deep brown, sweet-smelling solid residue left behind was weighed, stored in air- and water-proof container which was kept in a refrigerator at 4 ºC. This procedure was repeated for four more times. From this stock, fresh solutions were made whenever required.

$$\%\boldsymbol{Yield} = \frac{\text{weight of crude extract obtained (g)}}{\text{weight of starting pulverized dry leaves (g)}} \times \mathbf{100}\,\% \quad \ldots(\mathbf{1})$$

The average percentage yield of the *Ocimum gratissimum* L. ethanol leaf extract (*OG*) obtained was 11.52%.

2.4. Solvent fractionation of ocimum gratissimum linn. ethanol leaf extract (og)

OG was solvent-solvent portioned using procedure earlier described by Njan *et al.* [37] with slight modifications in the solvents and volumes used. Briefly described, *OG* solution was made with 150 ml of ethanol to which 250 ml of distilled water was added to give a hydroethanolic solution which was transferred into a 2L separating funnel. 1L of n-hexane was added and the mixture was rigorously shaken for 15 minutes to ensure complete dissolution. The solution was then left to stand for 4 hours to obtain two immiscible layers. The lower aqueous layer was gently drained into a clean container while the upper *n*-hexane fraction was drained into another container. This separation procedure was repeated for the hydroethanolic extract residue with further addition of n-hexane until a clear upper layer was obtained. The entire process was repeated with the extract residue using ethyl acetate followed by ethanol to obtain the ethyl acetate and ethanol fractions, respectively. The residue left behind, "marc", was kept in a clean container at the end of the experiment. Individual fraction obtained was concentrated *in vacuo* at 40 °C using the rotary evaporator using the process described by Adeneye *et al.* [46]. The concentrated fractions were further freeze-dried using a freeze drier (LTE LYOTRAP.LTE Scientific Ltd, Greenfield, England) and stored in both air- and water-tight containers and kept in the refrigerator at 4 °C as previously reported by Adeneye *et al.* [46]. The petroleum ether, ethyl acetate and ethanol fractions were denoted as *PEOG*, *EAOG* and *EOG*, respectively and used for the experiment.

2.5.Experimental animals

A total of seventy-five (75) of adult male Wistar Albino rats (aged 10-12 weeks old and body weight: 180-200 g) were obtained from the Animal Farm in Ogbomoso, Oyo State, Nigeria and housed in the Animal House of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria, after an ethical approval (UERC Approval number: UERC/ASN/2022/2327) was obtained from the University of Ilorin Ethical Review Committee for Staff and Postgraduate Research. The rats were acclimatized for 2 weeks in the facility, cared for and handled in line with global best practices guiding the Use and Handling of Experimental Animals as stipulated by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals [47]. Experimental rats were freely fed with standard rat feed and potable water and maintained at standard laboratory conditions throughout the period of the study. Prior to the commencement of the experiment, rats whose body weight differences within and between groups do not exceed ±20% of the average weight of all the experimental rats were randomly allotted to twelve (12) treatment groups and used for the study

2.6. Body weight measurement

The body weights of the experimental rats were taken at the beginning and end of the experiment using a digital rodent weighing scale (®Virgo Electronic Compact Scale, New Delhi, India) as previously described by Olorundare *et al.* [4, 45]. The values obtained were expressed in grams (g).

2.7. Experimental induction of *TZM*-induced cardio-toxicity and drug treatments

TZM-mediated cardiotoxicity was successfully induced in all the *TZM*-treated rats using the method earlier described by Olorundare *et al.* [4, 45] but with slight modifications. Briefly described, the experimental rats based on their treatment groups were orally pretreated with *OG*, *PEOG*, *EAOG*, *EOG* and VAL-LSP-VAL fixed dose combination therapy 1 hours prior to injection with 2.25 mg/kg of *TZM* given via the intraperitoneal route. This was done on daily basis for 7 consecutive days. The treatment protocol adopted is as follows:

DW + DW – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*
DW + *TZM* – 10 ml/kg/day distilled water *p.o.* + 2.25 mg/kg/day *TZM i.p.*
OG – 100 mg/kg/day *OG p.o.* + 1 ml/kg/day distilled water *i.p.*
OG + *TZM* – 100 mg/kg/day *OG p.o.* + 2.25 mg/kg/day *TZM i.p.*
PEOG – 100 mg/kg/day *PEOG p.o.* + 1 ml/kg/day distilled water *i.p.*
PEOG + *TZM* - 100 mg/kg/day *PEOG p.o.* + 2.25 mg/kg/day *TZM i.p.*
EAOG - 100 mg/kg/day *EAOG p.o.* + 1 ml/kg/day distilled water *i.p.*
EAOG + *TZM* - 100 mg/kg/day *EAOG p.o.* + 2.25 mg/kg/day *TZM i.p.*
EOG - 100 mg/kg/day *EOG p.o.* + 1 ml/kg/day distilled water *i.p.*
EOG + *TZM* - 100 mg/kg/day *EOG p.o.* + 2.25 mg/kg/day *TZM i.p.*
VAL-LSP–5mg/kg/day VAL-0.035 mg/kg/day LSP fixed-dose combination *p.o.* + 1 ml/kg/day distilled water *i.p.*
VAL-LSP+*TZM*–5mg/kg/day VAL-0.035 mg/kg/day LSP fixed-dose combination *p.o.* + 2.25 mg/kg/day *TZM i.p.*

The choice of 100 mg/kg/day of *OG* as the treatment dose was made based on the results of the preliminary studies previously conducted which showed it to be the most effective dose with the no toxicity.

2.8. Electrocardiography Measurement in treated rats

Electrocardiography was carried out using a 6-lead computer ECG machine using modified method of Adedapo *et al.* [48]. 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. 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 (HR), 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:

$$QT_c = \frac{QT}{\sqrt{RR.}} \quad \ldots(1)$$

3. Results and Discussion

3.1. Effect of *OG* and *OG* fractions on body weight gain pattern in *TZM*-treated rats

Daily treatment with DW+*TZM* administered intraperitoneally for 7 consecutive days did not produce any significant ($p>0.05$) average body weight changes in the treated rats when compared to the DW only-treated group (Table 1). Similarly, repeated oral pretreatments with *OG* and its fractions as well as VAL-LSP did not significantly ($p>0.05$) alter the average body weight change in the treated rats (Table 1). This shows that neither *TZM* treatment nor *OG* and its fractions pretreatments cause any remarkable changes in the weights of the treated rats. This result is at variance with our previous report that showed that intraperitoneal injection of *TZM* resulted in significant weight loss [4].

Table 1. Effect of Ocimum gratissimum Linn. leaf extract (OG), OG petroleum ether fraction (PEOG), OG ethyl acetate fraction (EAOG) and OG ethanol fraction (EOG) treatments on weight gain pattern in TZM-intoxicated rats

Treatment Group	Av. bwt. on: Day 1	%bwt. Day 7	changes
DW	216.00±28.97	202.40±72.22	04.49±07.49
DW+*TZM*	222.90±26.44	209.70±36.05	-03.22±04.62
OG+DW	211.40±21.71	212.60±20.20	-03.25±08.45
OG+*TZM*	217.20±33.53	216.50+33.10	-0.22±05.15
PEOG+DW	216.80±19.53	212.00±30.02	-01.92±14.09
PEOG+*TZM*	217.30±31.15	207.80±21.21	-03.19±12.17
EAOG+DW	215.30±23.42	216.90±21.20	0.91±03.76
EAOG+*TZM*	218.20±34.17	217.30±42.63	-0.72±04.20
EOG+DW	217.10±28.34	220.90±29.21	01.90 ± 05.79
EOG+*TZM*	218.10±16.65	221.40±13.33	-0.14±04.61
VAL-LSP+DW	218.70±17.54	223.10±20.63	02.00±03.52
VAL-LSP+*TZM*	216.30±23.22	206.30±18.17	-04.40±03.07

DW+DW – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*
DW+*TZM* – 10 ml/kg/day distilled water *p.o.* + 2.25 mg/kg/day *TZM i.p.*
OG+DW – 100 mg/kg/day *OG p.o.* + 1 ml/kg/day distilled water *i.p.*
OG+*TZM* – 100 mg/kg/day *OG p.o.* + 2.25 mg/kg/day *TZM i.p.*
PEOG+DW – 100 mg/kg/day *PEOG p.o.* + 1 ml/kg/day distilled water *i.p.*
PEOG+*TZM* – 100 mg/kg/day *PEOG p.o.* + 2.25 mg/kg/day *TZM i.p.*
EAOG+DW – 100 mg/kg/day EAOG *p.o.* + 1 ml/kg/day distilled water *i.p.*
EAOG+*TZM* – 100 mg/kg/day EAOG *p.o.* + 2.25 mg/kg/day *TZM i.p.*
EOG+DW – 100 mg/kg/day *EOG p.o.* + 1 ml/kg/day distilled water *i.p.*
EOG + *TZM* – 100 mg/kg/day *EOG p.o.* + 2.25 mg/kg/day TZM *i.p.*
VAL-LSP+DW – 5 mg/kg/day VAL-0.035 mg/kg/day LSP fixed-dose combination *p.o.* + 1 ml/kg/day distilled water *i.p.*
VAL-LSP+*TZM* – 5 mg/kg/day VAL-0.035 mg/kg/day LSP fixed-dose combination *p.o.* + 2.25 mg/kg/day TZM *i.p.*
VAL-LSP+*TZM* – 5 mg/kg/day VAL-0.035 mg/kg/day LSP fixed-dose combination *p.o.* + 2.25 mg/kg/day TZM *i.p.*

3.2. Effects of *OG* and *OG* fractions pretreatments on the ECG parameters in the *TZM*-intoxicated rats

The ECG changes caused by pre- and post-*TZM* treatments are shown in Tables 2 and 3, respectively. In this study, treatment with *TZM*-only was not associated with remarkable ECG changes (Table 3 and Figure 1b) when compared to the normal rats treated with distilled water only (Table 3 and Figure 1a). However, statistically significant ($p<0.05$) reductions in the QT and QTc values were observed in the treatment group administered with 5 mg/kg/day VAL- 0.035 mg/kg/day LSP fixed-dose combination only (Table 3 and Figure 1k) when compared with the untreated normal control and untreated *TZM*-intoxicated groups that received 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.* (Table 3 and Figure 1a) and 10 ml/kg/day distilled water *p.o.* + 2.25 mg/kg/day *TZM i.p.* (Table 3 and Figure 1b), respectively . Similarly, there was a significant ($p<0.05$) reduction in QT values in the group that was orally administered with 100 mg/kg/day *EOG* and 2.25mg/kg/day *TZM* (Table 3 and Figure 1j). QT interval is the time from the start of the Q wave to the end of the T wave on an ECG. It represents time taken for ventricular depolarisation and repolarisation, effectively the period of ventricular systole from ventricular isovolumetric contraction to isovolumetric relaxation [49]. The QT interval shortens and lengthens at faster heart rates and slower heart rates, respectively [50]. An abnormally prolonged QT is associated with an increased risk of ventricular arrhythmias, especially *torsades de pointes* while shortened QT interval has been reported to be associated with an increased risk for paroxysmal atrial and ventricular fibrillation and sudden cardiac death [51-53].

Although, reports on drug-induced QT-interval shortening are rare, new drugs are being

investigated for their ability to shorten action potential duration and/or QT interval as well as being profibrillatory [54]. In recent times, the arrhythmogenic potentials of QT interval shortening is now being highlighted in patients with short QT syndrome. Although the congenital forms of shortened QT interval is associated with arrhythmias and sudden cardiac death [55-56], the clinical relevance of drug-induced QT-interval shortening remains unclear [57]. Considering the fact that the rats that received the ethanol fraction of *Ocimun gratissimum* and distilled water (*EOG*+DW) had no statistically significant ($p<0.05$) differences in the QT-interval values (Table 3 and Figure 1i) when compared with the controls (Table 3 and Figure 1a), the additive effect of the combination of the ethanol fraction of *Ocimum gratissimum* and trastuzumab (*EOG*+*TZM*) probably led to the significant QT-interval shortening recorded in this study (Table 1 and Figure 1j). A shortened QT is reported to be associated with ventricular arrhythmia often which itself increases the risk of syncope or cardiac arrest [54, 58]. Thus, indicating that *EOG* and *TZM* co-treatment could be arrhythmogenic and result in sudden cardiac death. In a recent study, we reported the cardioprotective potential of *OG* and its fractions in *TZM*-intoxicated rats which was mediated via anti-apoptosis and and antioxidant mechanisms which were probably attributed the presence of secondary metabolites such as thymol, phenol, squalene, phthalic acid and neophytadiene that are present in *OG* and its fractions, *EAOG* and *EOG* in high amounts [59]. However, studies have shown phthalic acid (an ortho-carboxylic acid benzoic acid derivative) and its esters to be an endocrine disruptor and interfere with endogenous conversion of tryptophan to niacin in rats [60]. Other studies have shown that acute exposure to phthalate caused wide range of ECG abnormalities which include slowed atrioventricular conduction, increased atrioventricular node effective refractory period in an intact, whole heart model; while its prolonged exposure is associated with prolonged action potential duration time, enhanced action potential triangulation, and increased the ventricular effective refractory period; and slowed epicardial conduction velocity resulting from the inhibition of Nav1.5. [61-66]. Thus, the presence of this secondary metabolite and its ester, di(2-propylpentyl)ester, in high amount in *EOG* as shown through the GC-MS analysis conducted in the study [59] could be responsible for the arrhthmogenic effect of the *EOG* fraction. Also, the arrhythmogenic theory of *EOG* in this study appears to be corroborated by the histopathologic lesion of diffuse cardiomyocyte hypertrophy reported for the *EOG*-only treated and mild coronary artery tunica media thickening/hyperplasia recorded for the *EOG*-pretreated, *TZM*-intoxicated rats that we recently reported [59]. Combinations of valsartan, an angiotensin II receptor antagonist and lisinopril, an angiotensin converting enzyme inhibitor (ACEI) have been shown to improve clinical outcomes in patients with hypertension and microalbuminuria [67]. In an earlier study from our laboratory, a fixed-dose combination of valsartan and lisinopril showed promising results in the management of *TZM*-induced cardiotoxicity majorly mediated via antiapoptotic and antioxidant mechanisms in rats [4]. In this study, however, a combination of valsartan and lisinopril in a fixed dose caused a statistically significant ($p<0.05$) shortening of the QT/QTc values (Table 3 and Figure 1k). The shortening of the QT/QTc, when compared with the controls in this study might have been due to hyperkalemia, which is known to also shorten the QT interval. The shortening of the QT-interval in the valsartan and lisinopril combination group in this study might have been caused by hyperkalemia which is a known side-effect of the co-administration of an ACE inhibitor with an angiotensin II receptor antagonist [68]. However, the fact that the shortened QT/QTc-interval was not observed in the VAL-LSP pretreated *TZM*-intoxicated group (Table 3 and Figure 1l), this probably suggests that VAL-LSP fixed dose combination is safe for use in *TZM* chemotherapy. This result is in tandem with our previous study that showed VAL-LSP fixed dose combination ameliorated QT/QTc prolongation in *TZM*-intoxicated rats [69]. It is worthy to note that the EGC pattern was essentially normal in other treatment groups (Table 3, Figures 1c - 1h).

Table 2. Pretreatment (baseline) EGC parameters in the untreated Wistar rats on day 1 of the experiment

Treatments Groups	Heart Rate (/min)	P-duration (ms)	PR-Interval (ms)	QRS duration (ms)	QT segment (ms)	QT corrected	R amplitude (mV)
DW+DW	207.4±13.6	19.0±1.9	49.4±3.6	15.0±1.6	82.2±14.2	152.2±25.4	0.5 ± 0.1
DW+*TZM*	209.0±21.1	17.0±4.5	47.0±6.6	16.4±2.4	82.0±13.1	153.2±31.5	0.5±0.1
OG+DW	220.8±23.5	18.5±4.5	46.3±1.7	15.3±2.6	70.8 ± 6.0	128.0±17.5	0.5±0.1
OG+*TZM*	208.4±15.6	19.2±2.4	47.0±6.6	14.2±3.6	94.2 ± 7.0	175.4±18.7	0.4±0.2
PEOG+DW	213.0±6.2	20.3±1.5	49.7±2.1	14.3±3.1	86.0±3.5	161.7±7.6	0.5±0.1
PEOG+*TZM*	207.8±17.7	22.8±1.6	54.8±2.3	14.2±3.6	87.6±12.7	172.0±21.4	0.5±0.1
EAOG+DW	221.5±7.5	22.8±3.2	53.8±4.2	14.0±2.3	67.5±6.4	129.0±12.6	0.4±0.1
EAOG+TZM	224.8±10.7	19.5±4.4	44.3±1.7	17.5±1.3	76.8±12.7	153.8±27.4	0.5±0.2
EOG+DW	210.8±6.7	20.8±4.7	49.3±8.2	17.3±2.2	73.3±4.6	136.5±8.3	0.6±0.1
EOG+ TZM	219.3±13.5	23.0±3.9	48.8±5.9	13.5±2.4	78.5±10.8	149.0±16.2	0.4±0.2
VAL-LSP	221.8±17.7	20.2±4.1	49.8±1.9	15.6±2.6	72.0±8.9	137.8±15.6	0.5 ± 0.1
VAL-LSP+*TZM*	211.8±14.9	19.4±1.9	47.2±4.5	15.0±2.7	77.6±10.6	145.0±20.6	0.6 ± 0.2

Table 3. Effects of oral pretreatment with *OG* and *OG* fractions on the EGC parameters of *TZM*-intoxicated Wistar rats on day 7 of the experiment

Treatments Groups	Heart Rate (/min)	P-duration (ms)	PR-Interval (ms)	QRS duration (ms)	QT segment (ms)	QT corrected	R amplitude (mV)
DW+DW	199.0±20.5	22.0±2.0	48.5±6.6	16.5±6.5	95.5±17.5	171.8±40.5	0.4±0.1
DW+*TZM.*	201.3±26.4	19.0±1.8	49.0±1.4	16.0±1.4	97.0±21.4	178.3±44.8	0.4±0.1
OG+DW	209.0±11.6	17.5±3.9	51.3±3.6	13.3±2.1	77.3±12.7	144.0±26.4	0.4±0.1
OG+*TZM*	200.0±19.6	21.3±5.6	40.8±14.9	16.8±4.1	93.8±16.8	170.5±32.4	0.3±0.1
PEOG+DW	179.5±17.3	17.3±3.1	47.0±7.9	16.3±3.9	89.5±8.3	154.8±21.9	0.4±0.1
PEOG+TZM	200.3±41.0	26.3±9.0	56.5±8.5	15.5±4.0	79.0±16.1	144.3±38.6	0.4±0.2
EAOG+DW	184.3±26.4	22.8±4.7	50.3±3.9	14.8±3.4	93.0±3.6	162.0±9.3	0.5±0.1
EAOG+TZM	198.8±24.2	23.5±2.4	59.0±6.2	12.5±4.2	105.8±13.8	191.5±26.2	0.5±0.2
EOG+DW	220.8±16.6	18.8±2.5	45.3±3.2	13.3±1.3	81.3±14.8	156.0±33.0	0.5±0.1
EOG+ *TZM*	207.0±14.9	18.8±2.2	44.8±7.5	13.3±2.9	73.5±8.3*	135.8±11.8	0.5±0.2
VAL-LSP	193.3±20.9	18.5±2.9	49.5±3.4	16.5±3.1	70.0±7.7*	125.8±20.2*	0.5±0.1
VAL-LSP+*TZM*	184.0±13.0	18.3±3.4	50.0±2.9	13.8±1.5	87.8±13.5	152.8±21.2	0.5±0.1

* Statistically significant at $p<0.05$ when compared with the controls

Figure 1 (1a-1l). Showing the EGC changes associated with the oral *OG* and *OG* fractions pretreatments

4. Conclusions

In conclusion, results of this study showed that acute treatment with *TZM* was not associated with remarkable ECG changes and its oral pretreatments with *OG* and *OG* fractions (except *EOG*) may be safe. However, *EOG* administration may be arrhythmogenic in patients on *TZM* chemotherapy, therefore, considered unsafe as a cardioprotective adjuvant in *TZM* chemotherapy. However, further mechanistic studies will be required to provide better understanding of how *EOG* fraction causes QT shortening at the molecular level.

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