Anti-apoptotic and antioxidant mechanisms may underlie the abrogative potential of *Ocimum gratissimum* Linn. Leaf extract and fractions against trastuzumab-induced cardiotoxicity in Wistar rats

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ABSTRACT

Clinical use of trastuzumab (*TZM*), has been widely associated with increased incidence of cardiotoxicity. *Ocimum gratissimum* Linn. is a household medicinal plant popularly used for treating inflammatory conditions. In this study, we investigated the abrogative potential of 100 mg/kg/day of the ethanol leaf extract of *Ocimum gratissimum* Linn. (*OG*) and its petroleum ether (*PEOG*), ethyl acetate (*EAOG*) and ethanol (*EOG*) fractions in *TZM* intoxicated Wistar rats for 7 days using anthropometric, biochemical, histopathological and immunohisto-chemical endpoints. In addition, secondary metabolite constituents in *OG* and its fractions were determined through Gas Chromatography-Mass Spectrometry (GC-MS). The study results showed that oral pretreatments with *OG* and *OG* fractions as well as the fixed dose valsartan-lisinopril (VAL-LSP) combination effectively ameliorated and restore nearly normal levels the *TZM*-altered plasma cardiac troponin I and antioxidant profile which were corroborated by histopathological and immunohistochemical findings as indicated by the inhibition of *TZM*-induced activation of caspases-3 and − 9 and profound upregulation of BCL-2 expression. Phytoscan of *OG* and its fractions showed the presence of thymol and in high amount. Overall, our findings revealed the cardioprotective potentials of *OG*, *OG* fractions and fixed dose VAL-LSP combination against *TZM*-induced

cardiotoxicity which probably was mediated via abrogation of cardiomyocyte apoptosis and antioxidant mechanisms.

1. Introduction

Trastuzumab (*TZM*), since it was first approved by the Food and Drug Administration in September 1998 for the clinical management of patients with HER2-positive metastatic breast cancer, has remain the gold standard treatment strategy [48,61,99]. *TZM*, a purified recombinant DNA-based humanized monoclonal antibody, elicits its cytotoxic action by binding with high affinity and specificity to the extracellular domain IV of the tyrosine kinase human epidermal growth factor receptor 2 (HER2) expressed by solid tumors such as early and metastasized breast cancer [21,56], gastro-esophageal adenocarcinoma, gastric cancer and salivary gland tumors [33,52,83].

TZM induces anticancer action through multiple mechanisms that include HER2 internalization and degradation, antibody-dependent cellular cytotoxicity, and MAPK and PI3K/Akt interference [101,34, 97]. However, despite *TZM*'s high efficacy in cancer treatment, its use has been limited by its off-target cardiotoxicity and treatment resistance. *TZM* cardiotoxicity is postulated to be mediated via multiple mechanisms that include HER2 signaling dysregulation, cardiomyocyte autophagy suppression, and cardiomyocyte reactive oxygen species (ROS) accumulation [22,65] with all these culminating in accelerated cardiomyocyte apoptosis [65,66]. *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 [34,66]. To prevent cardiotoxicity, several strategies have been developed: (i) angiotensin-converting enzyme inhibitors, sartans, beta-blockers, aldosterone antagonists, and statins; (ii) iron chelators; and (iii) by development of new anthracycline derivatives with little or no cardiotoxicity [19], although their effectiveness remain debatable.

Ocimum gratissimum Linn. (*Lamiaceae*) is an aromatic perennial medicinal and ornamental plant that is widely distributed across tropical and subtropical regions of the world ([4,79] where it is commonly known as clove basil, African basil and wild basil [88]. It is a common food seasoning and condiment popularly used in the West African and Asian cuisines [4,40]. *Ocimum gratissimum* Linn. is known by different names and these include "Scent leaves" (Nigeria), "Nunu Bush" (Jamaica), "Fobazen" (Haiti), "Mujaaja" (Uganda) and "Maduruthala" (Sri Lanka) [70].

In the Asian traditional medicine, *Ocimum* especially *Ocimum basilicum* have the ancestral history of being used in the local treatment of heart diseases including hypertension and coronary heart disease [95]. Reports have it that *Ocimum gratissimum* Linn. possesses different biological activities and these include anti-stress, antidiarrheal [1,27,43, 72], anti-helminthic, anti-inflammatory, antipyretic [3,84,85], antioxidant and free radical scavenging [3,42], antimutagenic and anticancer [17], anti-ulcerative and gastroprotective, antibacterial [70], fungicidal [92], hepatoprotective [18], antiapoptotic [53]. Its other biological activities include male anti-fertility and aphrosidiac [69,71,74], and sedative activities [30,75]. Polyphenol-rich fraction of *Ocimum gratissimum* Linn. leaves have reportedly protected against cyclophosphamide-induced nephrotoxicity [5], gentamicin-induced hepatotoxicity [73] as well as lead acetate-induced cerebellar dysfunction [94] 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 [69]. While *Ocimum gratissimum* has been reported to possess antihyperlipidemic, anti-ischemic, and antihypertensive activities [91], a bioactive compound isolated from the plant leaves. gratissinol, is documented to be responsible for its cardioprotective activities [81].

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 [10,11]. Indeed, RV heart failure (RVHF) has been documented to co-exist with LVFH [14] and may be irreversible even after the discontinuation of chemotherapy unlike LVHF [44]. Other studies have demonstrated that *TZM* blocks the HER2 receptors in the cardiomyocytes rendering both RV and LV vulnerable to type II or reversible cardiotoxicity [64,93]. However, the thinner structure of the RV with lesser myofibrils may make it more vulnerable to the *TZM* toxicity [35].

In our quest to provide effective and affordable therapeutic option(s) for treating *TZM*-induced cardiotoxicity, this study was designed at evaluating the possible therapeutic potential of *Ocimum gratissimum* Linn. leaf extract and fractions in acute *TZM*-induced cardiotoxicity in Wistar rats, using biochemical markers (cardiac enzyme, complete lipids profile, cardiac oxidative stress, apoptosis), as well as histopathological and immunohistochemical endpoints.

2. Materials and methods

2.1. Drugs and chemicals

The drugs and chemicals used for this study are as reported by [77].

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 °C 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 h after which it was continuously stirred for 1 h 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.

%Yield was calculated as $= \frac{\text{weight of crude extract obtained(g)}}{\text{weight of starting pulverized dry leaf extracted(g)}} \times 100$

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. [69] 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 2 liter separating funnel. 1 liter of n-hexane was added and the mixture was rigorously shaken for 15 min to ensure complete dissolution. The solution was then left to stand for 4 h 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. [2]. 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. [2]. The petroleum ether, ethyl acetate and ethanol fractions were denoted as *PEOG*, *EAOG* and *EOG*, respectively and used for the experiment.

2.5. Gas chromatography-mass spectrophotometer (GC-MS) analysis of OG and OG fractions

The procedure for conducting the gas chromatography-mass spectrophotometer (GC-MS) analysis for *OG* and its solvent fractions to determine the relative abundance of its secondary metabolites was strictly in compliance with that earlier described by Olorundare et al. [76].

2.6. 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 [68]. 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.

2.7. 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. [77,78]. The values obtained were expressed in grams (g).

2.8. Experimental induction of TZM-mediated cardiotoxicity and drug treatments

TZM-mediated cardiotoxicity was successfully induced in all the *TZM*-treated rats using the method earlier described by Olorundare et al.

[77,78] 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 h 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:

Veh. – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*

Veh. + *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 – 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.*

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.

2.9. Blood sample collection

24 h after the last *TZM* injection, the overnight fasted rats were humanely sacrificed under controlled, light inhaled halothane anesthesia and whole blood samples were collected directly from the heart with fine 21 G injectable needle and 5 ml syringe. The rat heart was carefully identified, harvested and weighed.

2.10. Plasma cardiac troponin I and lipids measurements

Blood samples obtained via cardiac puncture was collected into 10 ml heparinized sample bottles and centrifuged at 5000 rpm to separate out the plasma that were used for the assays of cardiac troponin I (*cTnI*), triglyceride (TG), total cholesterol (TChol.) and cholesterol fractions: high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c)] using standard bioassay procedures and commercial kits.

2.11. Calculation of atherogenic and coronary artery risk indices (AI and CRI)

AI was calculated using the Castelli's risk indices:

$$LDL - c\left(\frac{mg}{dl}\right) \Big/ HDL - c\left(\frac{mg}{dl}\right)$$

and

$$LDL - c\left(\frac{mg}{dl}\right) \Big/ HDL - c\left(\frac{mg}{dl}\right)$$

while CRI was calculated as:

$$TCol.\left(\frac{mg}{dl}\right) \Big/ HDL - c\left(\frac{mg}{dl}\right)$$

as described by Lemieux et al. [54], Ren et al. [86] and Azeez [8].

2.12. Determination of cardiac tissue antioxidant profile

The cardiac tissue was obtained, preserved and the activities of oxidative stress markers such as superoxide dismutase (SOD), catalase

(CAT), glutathione-S-transferase (GST), Malondialdehyde (MDA) and reduced glutathione (GSH) were determined using methods earlier described by Olorundare et al. [78].

2.13. Histopathological studies of cardiac tissues

This was done using the right half of the heart dissected out and the choice of right ventricle being the most susceptible of the heart chambers to *TZM* toxicity [10,44]. Tissue slide preparations and readings were also done as earlier reported by Olorundare at al. (2021).

2.14. Enzyme-linked immunosorbent assay (ELISA) to determine cardiac tissue caspases-3 and − 9 levels

The levels of cardiac caspases-3 and − 9 were determined using the commercial enzyme linked immunosorbent assay (ELISA) kits (Wuhan Elabscience Biotechnology Company Limited, No. 1 Shizishan Street, Hongshan District, Wuhan, Hubei, China) following the Manufacturers' instruction and in line with the procedure earlier described by Olorundare et al. [78].

2.15. Immunohistochemical analysis of caspase-3 and BCL-2 expressions in cardiac tissues

The immunohistochemical staining of cardiac to determine the levels of expression of caspase-3 and BCL-2 as previously described by Olorundare et al., [78]. The immunoreactive positive expression of caspase-3 and BCL-2 indicated by brown staining on the prepared sample slides were viewed using a photo microscope (Olympus) and a digital camera (Toupcam®, China). Photomicrographs were analyzed for intensity of protein expression using image J Fiji software application.

2.16. Data analysis

Data were presented as mean ± S.E.M. of three observations for the immunohistochemical assays and mean ± S.D. of five observations for the in vivo studies. Statistical analysis was done using One-way analysis of variance followed by Tukey's post hoc test so as to compare within and between groups on GraphPad Prism Version 5. Statistical significance was considered at $p < 0.05$, $p < 0.001$, and $p < 0.0001$.

3. Results

3.1. Gas chromatography-mass spectrometry to determine secondary metabolites in OG, PEOG, EAOG and EOG fractions

GC-MS analysis of *OG* shows the presence and relative high abundance of thymol, β-caryophyllene, naphthalene, octahydronaphthalene, phenol isocaryophyllene naphthalene, neophytadiene, pentadecanoic acid, hexadecanoic acid ethyl ester, phytol, octadecatrienoic acid, octadecatrienoate, squalene and supraene although other secondary metabolites were also present but in relatively negligible amount {Table 1 and Fig. 1(a)}. The *PEOG* shows the presence of thymol, phenol esters, β-caryophyllene esters, guaiene, naphthalene esters, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 1,1′-di-n-butylferrocene, 4 H-1-benzopyran-4-one, methoxy-THC, phthalic acid esters, 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester and squalene {Table 2 and Fig. 1(b)}. The *EAOG* shows the presence and relative high abundance of cyclohexene, thymoquinone, thymol, phenol derivatives, N-ethyl-4-nitroaniline, 4-amino-1-butanol, 2,4-di-tert-butylphenol, p-cymene-2,5-diol, bicyclo[4.4.0]dec-1-ene, bicyclosequiphellandrene, neophytadiene, hexadecen-1-ol derivatives, phthalic acids, neophytadiene, tetradecanoic acid, 1-docosene, phytol, phthalate phthalic acid, octadecatrienoic acid and 1,3-benzenedicarboxylic acid amongst other secondary metabolites that in relatively low quantity in the fraction {Table 3 and Fig. 1(c)}. Similarly, *EOG* is abundant in 2(1 H)-

pyridinone, 6-hydroxy-1 H-pyrrole, benzofuran, 2,3-dihydrobenzofuran, 5-hydroxymethylfurfural, furan, 2,3-dihydro-5-methyl-2-butenal, 2,5-hexanediol, 2,5-dimethylpyrrolidine, 2-methoxy-4-trifluoromethyl-allopurinol, imidazole 2-trifluoromethyl-allopurinol, cycloheptasiloxane, 9-oxabicyclo[6.1.0]nonane isomers, hexadecenoic acid ethyl ester, octadecanoic acid ethyl ester, 9,12,15-octadecatrienoic acid esters, thymol, phenol esters, and phthalic acid ester {Table 4 and Fig. 1 (d)}.

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

Daily treatment with Veh. + *TZM* administered via intraperitoneal route for 7 consecutive days did not produce any significant ($p > 0.05$) average body weight changes in the treated rats when compared to the Veh.only-treated group (Table 5). Similarly, repeated oral pre-treatments 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 5).

3.3. Effects of OG and its fractions on the plasma lipids in TZM-treated rats

Treatment with Veh. + *TZM* caused significant ($p < 0.05$) increase in the LDL-c level (Fig. 2D) and this elevation was markedly lowered only in rats treated with VAL-LSP + *TZM* when compared to other *TZM*-treated groups (Fig. 2D). However, in the treatment groups on *OG*

Table 1
GC-MS Analysis showing the secondary metabolites and their relative abundance in *Ocimum gratissimum* Linn. ethanol leaf extract (*OG*).

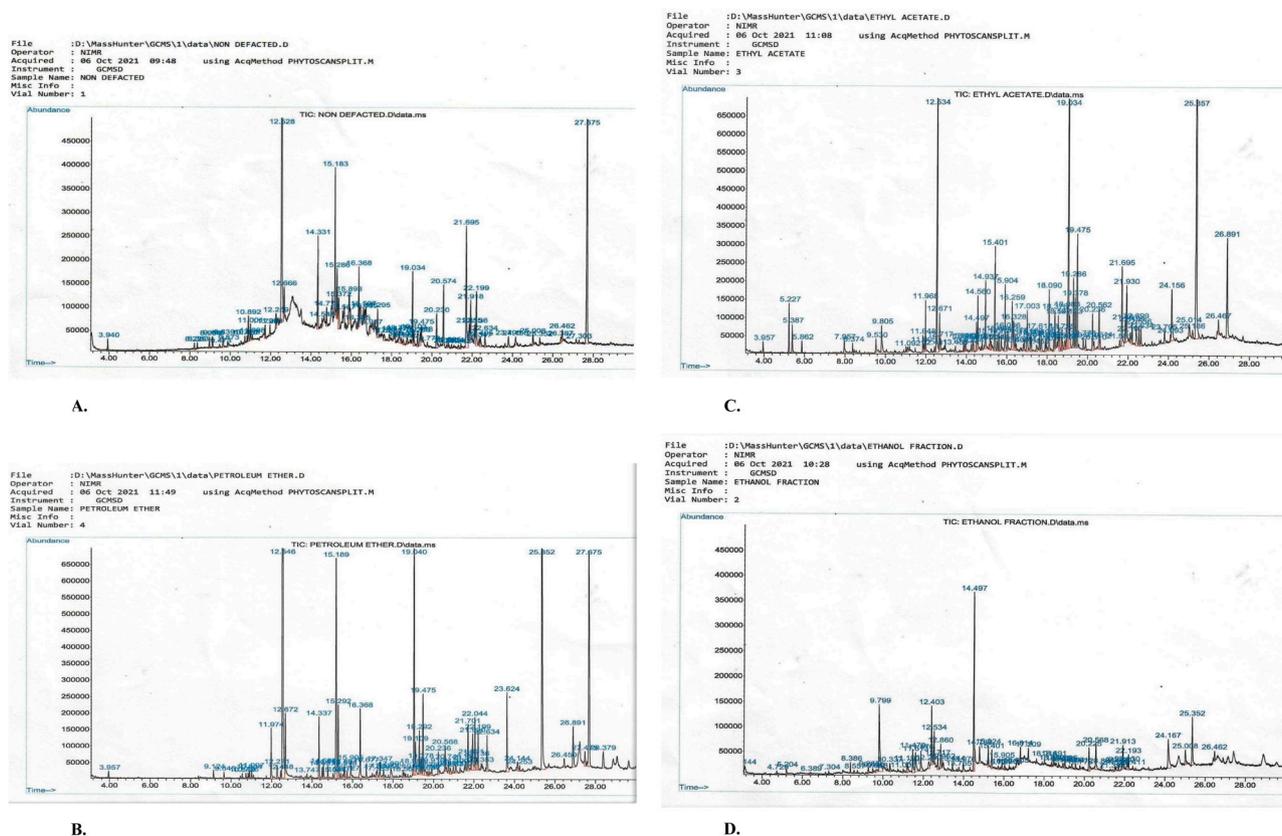| S/No.	RT Peak Area (%)	Sec. Metab. name	compound	CAS Library ID/Ref#	% Purity
1.	12.528	40.59	Thymol	000089-83-8	94
2.	14.331	2.65	β-Caryophyllene	000087-44-5	99
3.	15.183	4.07	Naphthalene	017066-67-0	99
4.	15.286	1.42	2-Isopropenyl-4a,8-dimethy-1, 2,3,4,4a,5,6,8a-octahydronaphthalene	1000193-57-0	98
5.	15.372	1.44	1,2,3,5,6,8a-hexahydro-4,7-dimethy-1-(1-methyl ethyl)	000483-76-1	90
6.	15.893	1.49	Phenol, 4-(methoxymethyl)− 2,6 dimethylbicyclo[4.3.0]nona-3,7-diene	005048-02-2	53
7.	16.368	1.64	isocaryophillenenaphthalene	1000140-07-2	90
8.	17.295	1.07	1.6-dimethyl-4-(1-methyl ethyl)-Naphthalene	000483-78-3	98
9.	19.034	1.89	Neophytadiene	000504-96-1	90
10.	20.230	1.29	Pentadecanoic acid	001002-84-2	91
11.	20.574	1.78	Hexadecanoic acid,ethyl ester	000628-97-7	97
12.	21.695	5.26	Phytol	000150-86-7	53
13.	21.918	1.41	9,12,15-octadecatrienoicacid	001191-41-9	91
14.	22.199	1.94	ethyl 9,12,15Octadecatrienoate	1000336-77-4	99
15.	27.675	13.51	Squalene	000111-02-4	99
			Supraene	007683-64-9	97

Fig. 1. GC-MS fingerprints/phytoscan showing the relative abundance of the secondary metabolites identifiable in *OG* (1 A), *PEOG* (1B), *EAOG* (1 C) and *EOG* (1D).

+ *TZM*, *PEOG* + *TZM*, *EAOG* + *TZM*, *EOG* + *TZM* and VAL-LSP + *TZM*, there were no significant (p > 0.05) alterations in the circulating levels of TG (Fig. 2A), TChol. (Fig. 2B), HDL-c lipoprotein (Fig. 2C), and VLDL-c lipoprotein fractions (Fig. 2E).

3.4. OG and OG fractions on the plasma AI and CRI in TZM-treated rats

The effects of *OG* and its fractions on the plasma atherogenicity (AI, HDL-c ÷ LDL-c ratio) and coronary artery index (CRI) in *TZM*-treated rats are depicted in Fig. 3(A)-3 C. Treatment with Veh. + *TZM* was associated with significant (p < 0.05) increases in the AI and CRI values (Figs. 3A and 3(C), respectively) while it caused a significant (p < 0.05) reduction in the HDL-c ÷ HDL-c ratio value (Fig. 3B). However, in the treatment groups on *PEOG* + *TZM*, *EAOG* + *TZM*, *EOG* + *TZM* and VAL-LSP + *TZM*, there were significant (p < 0.05) reversal and attenuation in the increase induced by Veh. + *TZM* treatment (Fig. 3A). On the CRI value, while Veh. + *TZM* caused a significant (p < 0.05) increase in its value, VAL-LSP + *TZM* treatment significantly attenuated the increase while *OG* and fractions did not significantly (p > 0.05) alter the CRI values (Fig. 3C). Similarly, *OG* and fractions did not significantly (p > 0.05) alter the HDL-c ÷ LDL-c ratio which was significantly (p < 0.05) decreased by Veh. + *TZM* treatment (Fig. 3B). However, this ratio was significantly (p < 0.05) increased by VAL-LSP + *TZM* treatment (Fig. 3B).

3.5. OG and OG fractions on the plasma cardiac troponin I (cTnI) levels in TZM-treated rats

Repeated daily treatments with Veh. + *TZM* caused a marked (p < 0.001) increase in the plasma *cTnI* when compared to the Veh.-only treated group (Table 6). However, oral pretreatments with *OG* and its fractions as well as fixed-dose VAL-LSP combination significantly attenuated (p < 0.05, p < 0.001) increases in the plasma *cTnI* level

when compared to the Veh. + *TZM*-treated group (Table 6).

3.6. OG and OG fractions on the cardiac tissue antioxidant activities in TZM-treated rats

Repeated Veh. + *TZM* treatment for 7 days caused significant (p < 0.001) decreases in the activities of cardiac tissue CAT, SOD, GST and GSH levels when compared to the Veh.only-treated group (Table 7). Conversely, repeated *TZM* treatment markedly (p < 0.05) increased MDA activity in the Veh. + *TZM* group when compared with Veh.only-treated group (Table 7). However, in groups pretreated with *OG* + *TZM*, *PEOG* + *TZM*, *EAOG* + *TZM*, *EOG* + *TZM* and VAL-LSP + *TZM*, there were significant (p < 0.05) restoration of the activities of the measured cardiac tissue antioxidant enzymes to near normal values (Table 7).

3.7. Histopathological studies of OG and OG fractions on the TZM-treated cardiac tissues

Treatments with Veh. + *TZM* for 7 days resulted in severe vascular congestion and cardiomyocyte degeneration and cartilaginous degeneration (Fig. 4B) when compared to normal cardiac histoarchitecture of Veh.-only treated group (Fig. 4A). These cardiac histopathological changes were markedly improved in *OG* + *TZM* (Fig. 4D), *EAOG* + *TZM* (Fig. 4H) and *EOG* + *TZM* (Fig. 4J), as well as VAL-LSP + *TZM* (Fig. 4L) treated groups. These improvements were essentially marked by normal cardiomyocytes with varying degree of coronary endothelial hyperplasia and cartilaginous degeneration. However, the *PEOG* + *TZM* treatment group was associated with diffuse myocardial scarring (Fig. 4F). In the groups treated with Veh. + *OG* (Fig. 4C), Veh. + *PEOG* (Fig. 4E), Veh. + *EAOG* (Fig. 4G), Veh. + *EOG* (Fig. 4I) and Veh. + VAL-LSP (Fig. 4K), there were no associated significant cardiac histological changes.

Table 2
GC-MS Analysis showing the secondary metabolites and their relative abundance in the petroleum ether fraction of *Ocimum gratissimum* Linn. leaf extract (*PEOG*).

S/No.	RT	Sec. Metab. Peak Area (%)	compound name	CAS Library ID/Ref#	% Purity
1.	12.546	46.07	Thymol	000089-83-8	94
			Phenol, 2-methyl-5(1-methylethyl)	000499-75-2	93
2.	14.337	1.09	β-Caryophyllene	000087-44-5	99
			Bicyclo[5.2.0]nonane	242794-76-9	99
			2-methylene-4,8,8-Trimethyl-4-vinyl-caryophyllene	017066-67-0	99
3.	15.189	4.85	Naphthalene, decahydro4a-methyl-1-methylene-7-(1-methylethyl)-[4aR-(4a.alpha.,7.alpha.,8a.beta)]-	017066-67-0	99
			Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4aR-(4a.alpha.,7.alpha.,8a.beta)].alpha.-Guaiene	003691-12-1	96
4.	15.292	1.38	2-isopropenyl-4a,8-dimethy-1,2,3,4,4a,5,6,8a-octahydro,naphthalene	1000193-57-0	83
			Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-[4aR-(4a.alpha.,7.alpha,8a.beta)]	017066-67-0	81
			2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalene	1000192-43-5	81
5.	16.368	1.40	Caryophyllene oxide	001139-30-6	80
6.	19.040	5.51	Neophytadiene	000504-96-1	94
7.	19.475	1.56	3,7,11,15-tetramethyl-2-hexadecen-1-ol methyl)− 2,6 dimethyl bicyclo[4.3.0]nona-3,7-diene	102608-53-7	72
8.	21.701	1.08	Phenol	000585-34-2	38
	-		m-tert-butyl-(z)− 2,6-dimethylocta 2,5,7-trien-4-one 2,5-diethylphenol	033746-71-3	38
				000876-20-0	35
9.	22.044	1.05	1,1′-di-n-butylferrocene	001274-08-4	46
			4 H-1-benzopyran-4-one	005128-44-9	43
10.	23.624	1.72	Methoxy-THC	1000380-01-1	64
11.	25.352	9.77	Bis(2-ethylhexyl) phthalate	000117-81-7	
			Phthalic acid, 2ethylhexyl isohexyl ester	1000308-98-5	90
12.	26.891	1.15	1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	000137-89-3	91

Table 2 (*continued*)

S/No.	RT	Sec. Metab. Peak Area (%)	compound name	CAS Library ID/Ref#	% Purity
13.	27.675	6.38	squalene	000111-02-4	

Table 3
GC-MS Analysis showing the secondary metabolites and their relative abundance in the ethyl acetate fraction of *Ocimum gratissimum* Linn. leaf extract (*EAOG*).

S/No.	RT	Sec. Metab. Peak Area (%)	compound name	CAS Library ID/Ref#	% Purity
1.	11.968	1.08	cyclohexene	000499-03-06	92
			Thymoquinone	000490-91-5	90
2.	12.534	8.66	Thymol	000089-83-8	94
			Phenol, 2-methyl-5-(1-methylethyl)	000499-75-2	93
3.	14.560	1.09	N-ethyl-4-nitroaniline	003665-80-3	72
4.	14.937	1.48	4-amino-1-butanol	100352-30-2	96
5.	15.401	2.05	2,4-di-tert-butylphenol	000096-76-4	97
6.	15.904	1.45	p-cymene-2,5-diol	002217-60-9	91
7.	16.259	1.33	Bicyclo[4.4.0]dec-1-ene	150320-52-8	95
8.	17.003	1.29	2-isopropyl-5-methyl-9-methylene-(+)-epi-Bicyclosesquiphellandrene.tau.	054274-73-6	92
9.	18.090	1.43	1-(1 S,3aR,4 R,7 S,7aS)− 4-Hydroxy-7isopropyl-4-methyl octahydro-1 H-inden-1-yl) ethanone	001911-78-0	98
10.	19.034	9.60	Neophytadiene	000504-96-1	91
11.	19.286	1.56	3.7,11,15-tetramethyl-2-hexadecen-1-ol	102608-53-7	68
12.	19.378	1.15	Phthalic acid	100356-84-3	
13.	19.475	2.46	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	102608-53-7	80
14.	20.236	1.06	Tetradecanoic acid	000544-63-8	86
15.	20.562	1.07	1-Docasene	001599-67-3	91
16.	21.695	2.27	Phytol	001599-67-3	91
17.	21.930	1.76	9,12,15-octadecatrienoic acid	000463-40-1	91
18.	24.156	1.97	3-methyl-4-isopropylphenol	003228-02-2	60
19.	25.351	28.45	Bis(2-ethylhexyl)phthalate	000117-81-7	91
20.	26.891	3.42	Phthalic acid 1,3-benzenedicarboxylic acid	000137-89-3	62

Table 4

GC-MS Analysis showing the secondary metabolites and their relative abundance in the ethanol fraction of *Ocimum gratissimum* Linn. leaf extract (*EOG*).

S/No.	RT	Sec. Metab. Peak Area (%)	compound name	CAS Library ID/Ref#	% Purity
1.	3.144	1.01	5-(prop-2-enoyloxy)pentadecane	1000245-67-6	35
2.	9.799	9.96	2(1 H)-pyridinone 6-hydroxy-1 H-pyrrole	000626-06-2	72
3.	11.190	1.16	catechol	000120-80-9	87
4.	11.470	2.79	benzofuran 2,3-dihydrobenzafuran	000496-16-2	87
5.	11.613	2.85	5-hydroxymethylfurfural	000067-47-0	64
6.	11.876	2.64	furan 2,3-dihydro-5-methyl-2-butenal	001487-15-6	64
7.	12.260	1.12	2-furancarboxaldehyde 5-methyl-2-furancarboxaldehyde	000620-02-0	30
8.	12.403	4.71	2,5-hexanediol	000110-03-2	53
9.	12.534	2.39	Thymol 3-methyl-4-isopropylphenol	000089-83-8 003228-02-2	93 53
10.	12.717	1.42	1,1-dimethyl-1-silacyclo-3-pentene Thiophene 2-ethyl thiophene 3-ethyl thiophene	016054-12-9 001795-01-3 001795-01-3	43 43 43
11.	12.860	2.02	2-ethoxy-4-vinylphenol	007786-61-0	87
12.	13.976	1.16	1,3-disilacyclobutane	001627-98-1	38
13.	14.497	16.85	Imidazole,2-trifluoromethyl-allopurinol	066675-22-7	53
14.	14.789	1.71	benzene,1,2-dimethyl-4-nitrobenzenemethanol	000099-51-4	60
15.	15.224	2.07	cycloheptasiloxane	000107-50-6	90
16.	15.401	1.77	phenol	000128-39-2	94
2,6-bis(1,1-dimethylethyl) phenol	000128-39-2	92			
17.	16.814	1.87	1-(4-tert-butylphenyl)propan-2-one	081561-77-5	35
18.	17.209	1.32	N-(trifluoroacetyl)− 0,0′,0″-tris trimethylsilyl)norepinephrine	1000072-26-3	47
19.	20.225	2.62	9-oxabicyclo[6.1.0]nonane	000286-62-4	25
20.	20.568	2.58	hexadecanoic acid, ethyl ester	000628-97-7	89
21.	21.913	2.53	9,12,15-octadecatrienoic acid (z,z,z)− 9,12,15-octadecatrienoicacid, methyl ester	000463-40-1 000463-40-1	91 91
22.	22.193	1.36	bicyclo[4.3.0]nonane,2-methylene	040954-37-8	62
23.	24.167	5.50	Thymol Phenol,2,3,4,6-tetramethyl-phenol Phenol,2-methyl-5-(1-methylethyl)	000089-83-8 003238-38-8 000499-75-2	90 87 87
24.	25.008	1.82	Myristoyl chloride Undecanoyl chloride Octadecanedioic acid	000112-64-1 017746-05-3 000871-70-5	49 43 43
25.	25.352	5,26	Phthalic acid, di(2-propylpentyl)ester	1000377-93-5	90
26.	26.462	1.00	cyclooctane		002213-60-7

3.8. Immunohistochemical studies of OG and OG fractions on TZM-treated cardiac tissues

3.8.1. OG and OG fractions on TZM-treated cardiac tissue caspase-3 and capsase-9 levels

Using ELISA technique, Veh. + TZM treatment caused marked (p < 0.05) elevation in the total cardiac caspase-3 and caspase-9 levels when compared with Veh. only-treated (untreated normal) cardiac tissue (Figs. 5A and 5(B), respectively). Treatments with Veh. + OG, Veh. + PEOG, Veh. + EAOG, Veh. + EOG and Veh. + VAL-LSP was associated with no significant (p > 0.05) changes in the cardiac tissue caspase-3 levels (Fig. 5A). However, with EAOG + TZM and EOG + TZM treatments, there were significant (p < 0.05) attenuation in the elevated caspase-3 levels (Fig. 5A). Similarly, in groups treated with OG + TZM, PEOG + TZM, EAOG + TZM, EOG + TZM and VAL-LSP + TZM, there was profound (p < 0.05) decreases in caspase-9 levels when compared with the Veh. + TZM-treated values (Fig. 5B).

3.8.2. OG, OG fractions and VAL-LSP fixed dose combination on TZM-treated cardiac tissue caspase-3 expression and intensity score

Caspase-3 expressions in the TZM intoxicated cardiac tissues and cardiac tissues subsequently pretreated with OG, PEOG, EAOG, EOG and VAL-LSP fixed dose combination are depicted in Fig. 6. Veh. + TZM-treated cardiac tissue showed enhanced expression of caspase-3 when compared with Veh. group (Fig. 6 A). Quantification of the immunohistochemical intensity showed profound (p < 0.05) increase in intensity score suggesting profound enhanced caspase-3 expression in the Veh. + TZM-treated cardiac tissue compared with the Veh.only-treated group (Fig. 6B). However, there were substantial (p < 0.05) decreases in intensity scores for the groups treated with OG +TZM, PEOG +TZM, EAOG + TZM, EOG +TZM and VAL-LSP +TZM (Fig. 6B).

3.8.3. OG, OG fractions and VAL-LSP fixed dose combination on TZM-treated cardiac tissue BCL-2 expression and intensity scoring

The immunohistochemical staining for BCL-2 expression and intensity scores for the treated cardiac tissues are depicted in Fig. 7 A and 7B, respectively. Veh. + TZM treatment caused a statistically non-significant (p>0.05) reduction in the BCL-2 intensity score (Fig. 7B) while in the OG + TZM, PEOG + TZM, EAOG + TZM, EOG + TZM and VAL-LSP +TZM treatment groups, the BCL-2 expression was not significantly (p>0.05) improved (Fig. 7B). Similar patterns were recorded in the BCL-2 expressions in Fig. 7 A.

4. Discussion

Experimental induction of drug-induced cardiotoxicity with

Table 5

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 Groups	Average body weights on:		%weight changes
	Day 1	Day 7	
Veh.	216.00 ± 28.97	202.40 ± 72.22	04.49 ± 07.49
Veh. + TZM	222.90 ± 26.44	209.70 ± 36.05	-03.22 ± 04.62
OG	211.40 ± 21.71	212.60 ± 20.20	-03.25 ± 08.45
OG + TZM	217.20 ± 33.53	216.50 ± 33.10	-00.22 ± 05.15
PEOG	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	215.30 ± 23.42	216.90 ± 21.20	00.91 ± 03.76
EAOG + TZM	218.20 ± 34.17	217.30 ± 42.63	-00.72 ± 04.20
EOG	217.10 ± 28.34	220.90 ± 29.21	01.90 ± 05.79
EOG + TZM	218.10 ± 16.65	221.40 ± 13.33	-00.14 ± 04.61
VAL-LSP	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

Veh. – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*

Veh. + 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 – 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.*

repeated administration of *TZM* is well reported [26,51,58,77]. In this study, repeated daily treatment of rats with 2.25 mg/kg of *TZM* administered via the intraperitoneal route for 7 consecutive days successfully established cardiotoxicity which was positively associated with profound elevation in the plasma *cTnI*. Generally, *cTnI* is considered to be a reliable and specific marker of acute myocardial injury due to its confinement and specificity to cardiac muscles [63,82,9]. Thus, myocardial injury from whatever cause is often associated with cardiomyocyte necrosis with attendant rise in the blood *cTnI* and cardiac troponin T (*cTnT*) levels [49,6,60]. However, there are emerging evidences that there could be increased blood levels of these cardiac enzyme markers without corresponding cardiomyocyte necrosis and apoptosis, usually from cardiac perfusion ischemia [32,36,38,98]. Of these two mechanisms, the former appeared to be the likely mechanism by which *TZM* mediated its cardiotoxicity in our study as evidenced by the profound increase in the plasma *cTnI* levels and cardiomyocyte and cartilaginous degeneration. Also, the fact that there was an increased cardiac tissue caspase-3 protein expressions coupled with reduced SOD and CAT activities as well as the accumulation of GSH and MDA levels following repeated intraperitoneal injections of *TZM*, all showed that *TZM*-induced cardiotoxicity was fully established in this experiment. However, these biochemical, immunohistochemical and histopathological alterations were effectively abrogated by oral pretreatments with *OG* and *OG* fractions, especially the *EAOG* fractions which exhibited the most effective abrogation.

TZM is notorious for inducing accelerated cardiomyocytes apoptosis which is marked by the upregulation of cardiac tissue caspase-9 and caspase-3 expressions while down-regulating the cardiac tissue BCL2 expression [13,57,62]. In the present study, we observed that repeated *TZM* treatment was similarly associated with increased caspases-9 and − 3 expressions indicating cardiomyocyte apoptosis in line with previous reports although the BCL2 expression was unaltered. The reason for
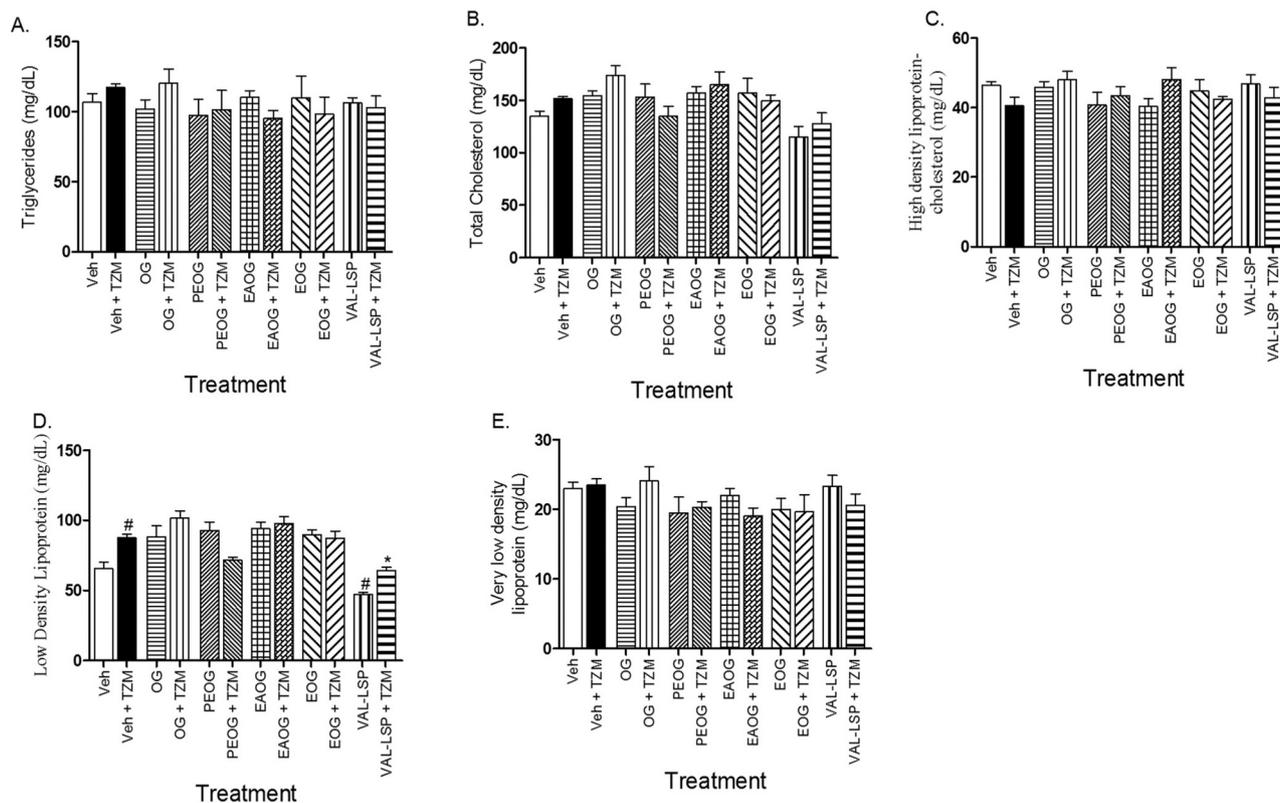

Fig. 2. Depicts the effect of oral treatments with *TZM, OG, PEOG, EAOG* and *EOG* on plasma TG **(2 A)**, TChol. **(2B)**, HDL-c **(2 C)**, LDL-c **(2D)** and VLDL-c **(2E)** in *TZM*-intoxicated rats. Bar represents mean ± SEM (n = 5), significant difference denoted by [#]$p < 0.05$ vs Group I or [*]$p < 0.05$ vs Group II by one-way ANOVA followed by Tukey's post hoc test.
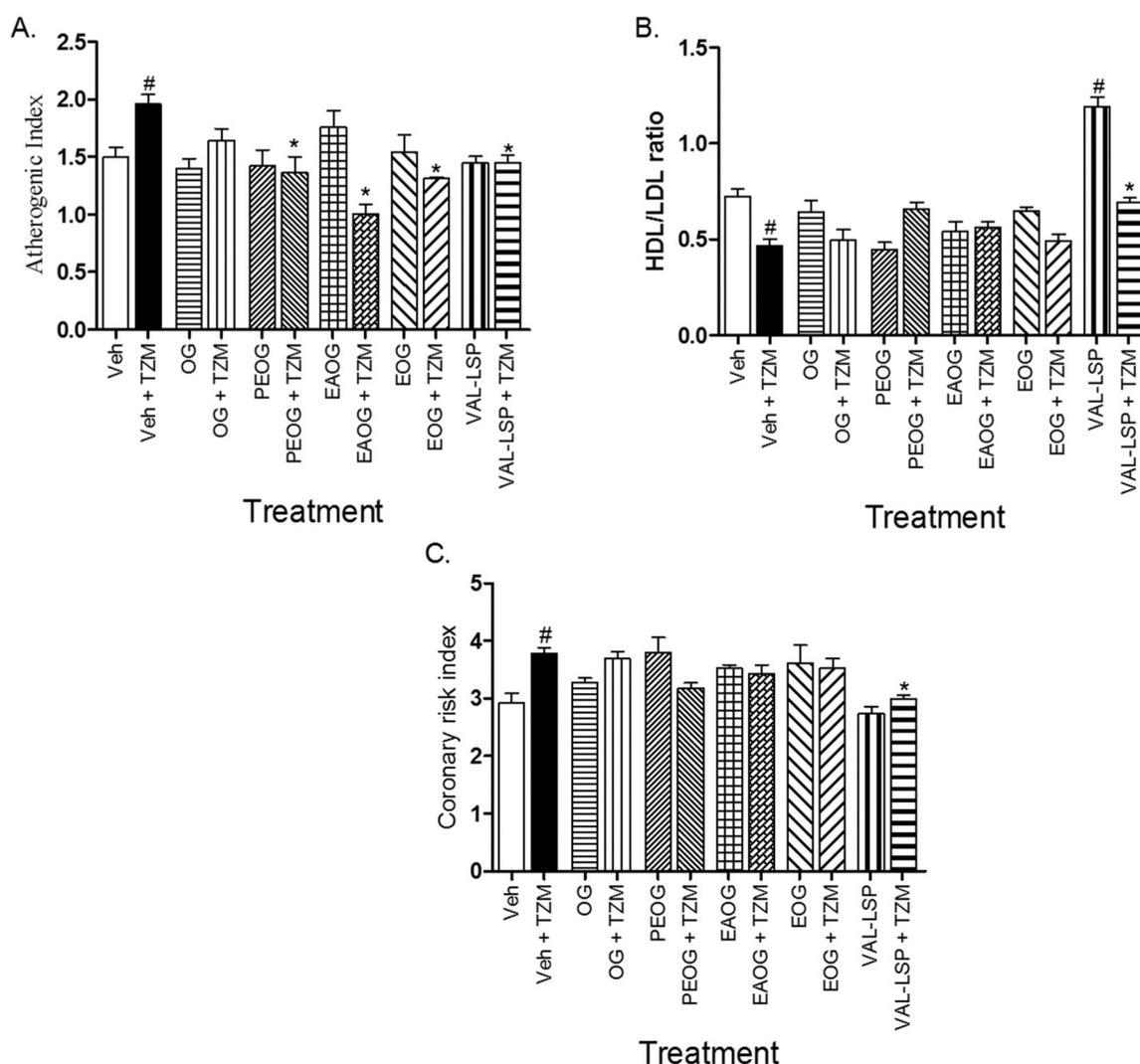
Fig. 3. Effect of *OG* and fractions on plasma atherogenicity {AI **(3 A)** and HDL-c ÷ LDL-c ratio **(3B)**} and plasma coronary artery index (CRI) **(3 C)** in *TZM*-treated rats. Bar represents mean ± SEM (n = 5), significant difference denoted by [#]$p < 0.05$ vs Veh.only-treated group or *$p < 0.05$ vs Veh. + *TZM*-treated group by one-way ANOVA followed by Tukey's post hoc test.

this variant effect on the BCL2 expression in our present study remains unknown but this could constitute an area of further studies in the nearest future. However, oral pretreatment with *OG* and *OG* fractions effectively reversed the *TZM* -mediated caspases-9 and − 3 upregulation without altering cardiac tissue BCL-2 expression suggesting anti-apoptosis mechanism as a possible cardioprotective mechanism of *OG* and its fractions.

Increases in TChol, TG, and LDL-c and reduction in HDL-c are reliable key cardiovascular disease risk predictors which could be elevated in various heart diseases including drug (*TZM*)-mediated cardiotoxicity [8,15,50,55]. The fact that there were attenuations in the *TZM*-induced elevations in the plasma AI by the oral pretreatments with *OG* and *OG* fractions suggested the possible cardioprotective potential of these bioactive agents in *TZM* cardiotoxicity. Also, the fact that *EAOG* and *EOG*, profoundly lowered the CRI is indicative of their cardioprotective potentials. Similar cardioprotective potential was offered by VAL-LSP combination and this is in strong support of the earlier report of the cardioprotective potential of VAL-LSP combination against *TZM*-mediated cardiotoxicity [78].

The presence of secondary metabolites such as thymol, phenol, squalene, phthalic acid and neophytadiene that are present in *OG*, *EAOG* and *EOG* in high amounts could be responsible for the observed protective effects and mechanisms against *TZM*-mediated cardiotoxicity,

although this remains speculative. These secondary metabolites are known to elicit various biological effects and these include anti-hyperglycemic and antihyperlipidemic [90], anti-obesity [37], antihypertensive and vasorelaxant [67,89], gastroprotective [31,87], hepatoprotective [31], nephroprotective [24,46], neuroprotective [47], antioxidant, free radicals scavenging, anti-inflammatory and anti-apoptosis activities [23,80], and antiparasitic [39] effects.

Thymol, chemically known as 2-isopropyl-5-methylphenol, is a colorless crystalline dietary monoterpene phenol with diverse biological actions that include anti-oxidative [23,67], antihyperlipidemic and anti-atherosclerotic effects [45,100], anti-apoptotic and anti-inflammatory [12,29]. Thymol and carvacrol were also reported to have exhibited synergistic cardioprotective effect against doxorubicin-induced cardiotoxicity that was attributed to their antioxidant, anti-inflammatory, and antiapoptotic activities [25]. Although, both carvacrol and thymol share the same chemical formula ($C_{10}H_{14}O$) and appear closely similar even with their *m/z* value on GC-MS because they are isomers. However, the key difference lies in the fact that carvacrol contains a hydroxyl group at the *ortho* position of the benzene ring whereas thymol contains a hydroxyl group at the *meta* position of the benzene ring. Therefore, thymol is more hydrophobic than carvacrol [16, 20]. Equally, squalene has been documented to elicit cardioprotective effect via inhibition of lipids accumulation and

Table 6

Effect of *Ocimum gratissimum* Linn. leaf extract (*OG*), *OG* petroleum ether fraction (*PEOG*), *OG* ethyl acetate fraction (*EAOG*), *OG* ethanol fraction (*EOG*) and valsartan-lisinopril fixed dose combination on the plasma cardiac troponin I (*cTnI*) levels in *TZM*-intoxicated rats.

Treatment Groups	Plasma cardiac troponin I levels (ng/ml)
Veh.	04.62 ± 01.35
Veh. + TZM	13.21 ± 01.80[c+]
OG	01.77 ± 00.84[c-]
OG + TZM	08.85 ± 04.51[a+]
PEOG	03.99 ± 01.07[a-]
PEOG + TZM	02.86 ± 01.87[a-]
EAOG	03.18 ± 00.93[a-]
EAOG + TZM	02.32 ± 01.21[c-]
EOG	01.75 ± 00.36[c-]
EOG + TZM	02.16 ± 01.23[a-]
VAL-LSP	01.39 ± 00.26[c-]
VAL-LSP + TZM	01.46 ± 00.51[c-]

[c+] represents a significant increase at $p < 0.05$ when compared to values for the Veh.only-treated group while [a-] and [c-] represent significant decreases at $p < 0.05$ and $p < 0.001$ when compared to values for the Veh. + *TZM*-treated group
Veh. – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*
Veh. + 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 – 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.*

antioxidant [28], anti-inflammatory [41], anti-atherosclerotic and antineoplastic [59] mechanisms. The fact that thymol was abundantly present in *OG*, *EAOG* and *EOG* as well as the fact that thymol has antioxidant and anti-apoptosis activities acting alone or in combination with other secondary metabolites strongly suggest it could be the active principle in *OG* and its fractions responsible for the observed anti-apoptosis observed in this study, although the *EAOG* appeared to be most effective in this respect going by its remarkable inhibition of the cardiac tissue caspase-3 and caspase-9 expressions. Similarly, *OG* and its fractions, especially *EAOG* are relatively abundant in β-caryophyllene, a finding that is similar to that previously reported by Olugbade et al. [79]. β-caryophyllene, a dietary phytocannabinoid sequiterpene, has previously been reported to attenuate oxidative stress, inflammation, apoptosis and prevents structural alterations of the myocardium against doxorubicin-induced acute cardiotoxicity in both in vitro and in vivo studies [7]. Thus, the presence of this secondary in high relative abundance in *OG* and *PEOG* could also be responsible for the recorded anti-apoptotic and antioxidant activities in the present study. β-caryophyllene was also reported to inhibits Fas-receptor (FasR) and caspase-mediated apoptosis signaling pathway and endothelial dysfunction in experimental myocardial injury [96]. Thus, it is plausible that β-caryophylline in *OG* and *PEOG* was responsible either in part or wholly for the extracts' cardioprotective effects against *TZM*-cardiotoxicity via these same mechanisms.

In conclusion, this study has highlighted the promising therapeutic potentials of *OG* and its fractions (especially *EAOG* fraction) in ameliorating *TZM*-induced cardiotoxicity that were probably mediated via anti-apoptosis and antioxidant mechanisms. However, bioassay-guided isolation and structural elucidation of the bioactive compounds in each of the *OG* fractions will be required in the near future.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Olufunke Esan OLORUNDARE reports financial support was provided by Tertiary Education Trust Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in
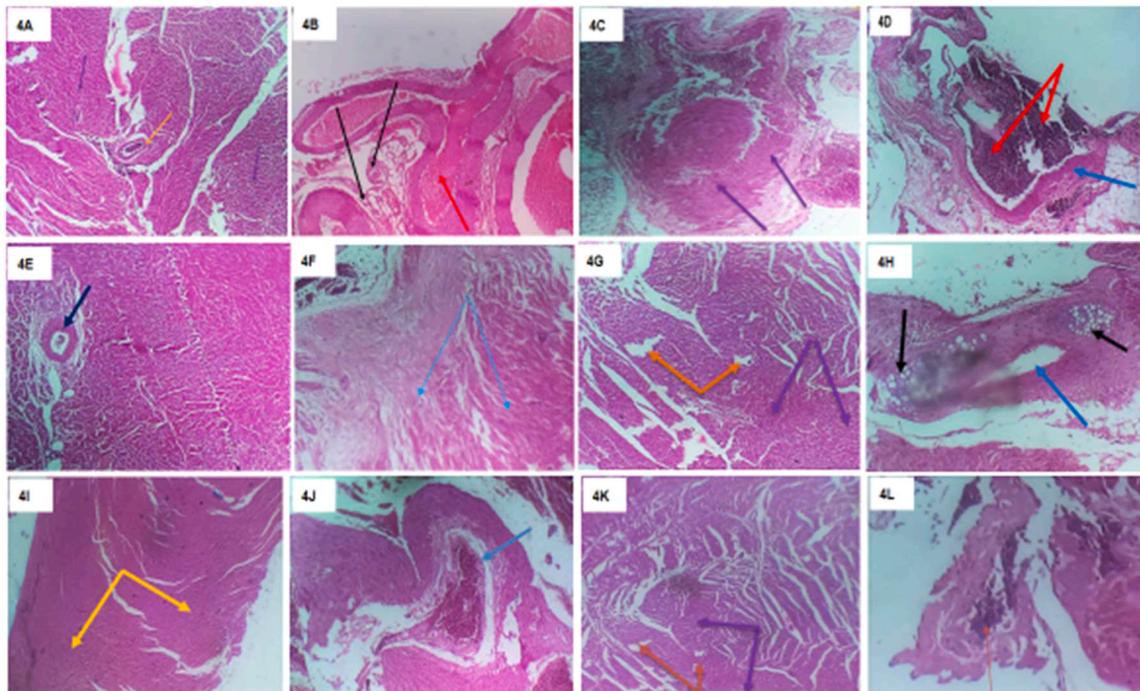
Table 7

Effect of *Ocimum gratissimum* Linn. leaf extract (*OG*), *OG* petroleum ether fraction (*PEOG*), *OG* ethyl acetate fraction (*EAOG*), *OG* ethanol fraction (*EOG*) and valsartan-lisinopril (*VAL-LSP*) fixed dose combination on the cardiac antioxidant enzyme activities in *TZM*-treated Wistar rats.

Treatment Groups	GSH (μmoles/mg protein)	MDA (ηmoles/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)	GST (U/min/mg protein)
Veh.	63.58 ± 1.56	13.25 ± 0.61	40.20 ± 0.73	1.42 ± 0.04	0.53 ± 0.04
Veh. + TZM	25.57 ± 1.22#-	27.87 ± 0.74#	16.97 ± 1.19#-	0.75 ± 0.08#-	0.23 ± 0.05#-
OG	40.58 ± 1.83 *	13.64 ± 0.58 * -	31.17 ± 1.77 *	1.18 ± 0.04 *	0.51 ± 0.03 *
OG + TZM	38.32 ± 0.92 *	15.96 ± 0.56 * -	28.51 ± 1.34 *	1.32 ± 0.05 *	0.42 ± 0.03 *
PEOG	47.94 ± 3.50 *	17.93 ± 1.36 * -	27.54 ± 1.11 *	1.48 ± 0.10 *	0.48 ± 0.07 *
PEOG + TZM	44.19 ± 2.12 *	17.56 ± 2.76 * -	26.61 ± 2.34 *	1.60 ± 0.16 *	0.41 ± 0.08 *
EAOG	42.41 ± 1.26 *	15.07 ± 0.44 * -	25.57 ± 0.41 *	1.38 ± 0.08 *	0.44 ± 0.05 *
EAOG + TZM	39.31 ± 1.07 *	17.12 ± 1.08 * -	23.73 ± 0.44 *	1.78 ± 0.11 *	0.44 ± 0.02 *
EOG	39.73 ± 2.57 *	14.45 ± 0.42 * -	28.23 ± 1.49 *	1.45 ± 0.08 *	0.51 ± 0.06 *
EOG + TZM	32.97 ± 1.68 *	16.82 ± 0.96 * -	25.12 ± 1.48 *	1.56 ± 0.20 *	0.48 ± 0.05 *
VAL-LSP	39.92 ± 1.11 *	14.30 ± 0.63 * -	27.28 ± 0.44	1.44 ± 0.37 *	0.45 ± 0.07 *
VAL-LSP+ TZM	38.82 ± 1.99 *	18.72 ± 0.79 * -	25.75 ± 2.03 *	1.69 ± 0.14 *	0.41 ± 0.03 *

Values represents mean ± SEM (n = 5)
[#] and [#-]represents a significant increase and decrease at $p < 0.05$, respectively, when compared to values for the Veh.only-treated group while [*] and [*-] represent a significant increase and decrease at $p < 0.05$, respectively, when compared to values for the Veh. + *TZM*-treated group.
Veh. – 10 ml/kg/day distilled water *p.o.* + 1 ml/kg/day distilled water *i.p.*
Veh. + 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 – 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.*

Fig. 4. A representative photographic section of (i). Veh.only-treated cardiac tissue showing normal coronary vasculature (indicated in brown thick arrow) and normal cardiomyocytes (indicated in purple thick arrows) (x100 magnification, Hematoxylin & Eosin stains) **(4 A)**; (ii). Veh. + *TZM*-treated rat cardiac tissue showing myocardiocyte degeneration with cartilaginous degeneration (indicated in black thick arrow) and severe coronary arteriolar congestion indicated in red thick arrow (x100 magnification, Hematoxylin & Eosin stains) **(4B)**; (iii). *OG*-only pretreated cardiac tissue showing normal cardiomyocytes (indicated in purple thick arrows) (x100 magnification, Hematoxylin & Eosin stains) **(4 C)**; (iv). *OG + TZM*-treated rat cardiac tissue showing moderate coronary arteriolar congestion and coronary artery tunica media thickening indicated by red thick and blue thick arrows, respectively (x100 magnification, Hematoxylin & Eosin stains) **(4D)**; (v). *PEOG*-only pretreated rat cardiac tissue showing coronary artery tunica media hyperplasia resulting in its narrowing (x100 magnification, Hematoxylin & Eosin stain) **(4E)**; (vi). *PEOG + TZM*-treated rat cardiac tissue showing myocardial scarring (indicated in blue thin arrow) (x400 magnification, Hematoxylin & Eosin stain) **(4 F)**; (vii). *EAOG*-only pretreated cardiac tissue showing normal coronary vessels and cardiomyocytes (x100 magnification, Hematoxylin & Eosin stains) **(4 G)**; (viii). *EAOG + TZM*-treated rat heart tissue showing coronary artery tunica media hyperplasia and cartilaginous deposits in blue thick and black thick arrows, respectively (x100 magnification, Hematoxylin & Eosin stains) **(4 H)**; *EOG*-only pretreated cardiac tissue showing diffuse cardiomyocyte hypertrophy indicated in yellow thick arrow (x100 magnification, Hematoxylin & Eosin stains) **(4I)**; *EOG + TZM*-treated rat heart tissue showing mild coronary artery *tunica media* thickening/hyperplasia indicated in blue thick arrow (x100 magnification, Hematoxylin & Eosin stains) **(4 J)**; VAL + LSP fixed dose combination-only pretreated cardiac tissue showing normal coronary vessels (indicated in the brown thick arrows) and normal cardiomyocytes (indicated in purple thick arrows) ((x100 magnification, Hematoxylin & Eosin stains) **(4 K)**; VAL-LSP fixed dose combination + *TZM*-treated rat heart tissue showing coronary artery cystic medial degeneration indicated in orange thin arrow (x400 magnification, Hematoxylin & Eosin stains) **(4 L)**.
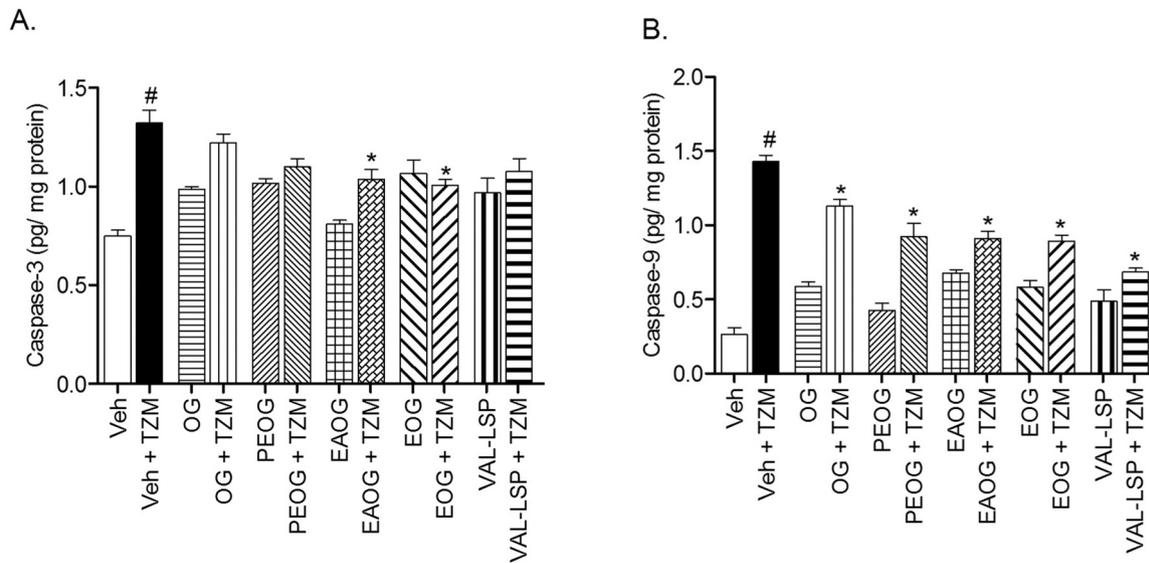

Fig. 5. Effect of oral pretreatment with *OG, PEOG, EAOG* and *EOG* on cardiac caspase-3 **(5 A)** and cardiac caspase-9 **(5B)** in *TZM*-intoxicated rats.
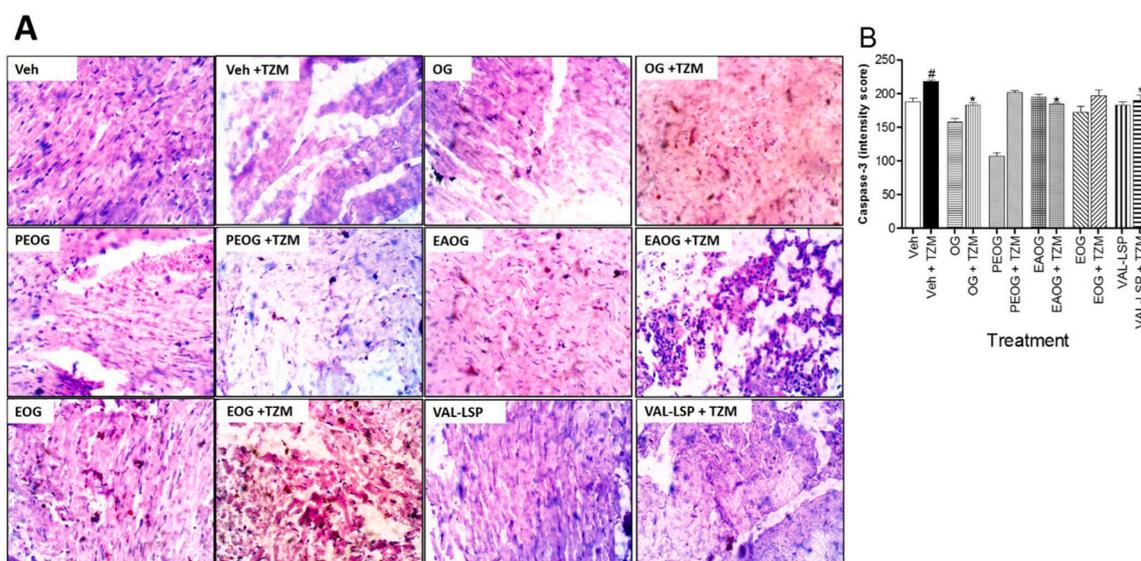
Fig. 6. . (A) Representative section of photomicrographs of immunohistochemical staining of cardiac tissue caspase-3 expression of cardiac tissues pretreated with *OG* and fractions (magnification x 400), and (B) Intensity score of expressions. Bar represents mean $\pm$ SEM (n = 3), significant difference denoted by [#]p < 0.05 vs Veh.only-treated group or *p < 0.05 vs Veh + *TZM*-treated group by One-way ANOVA followed by Tukey's post hoc test.
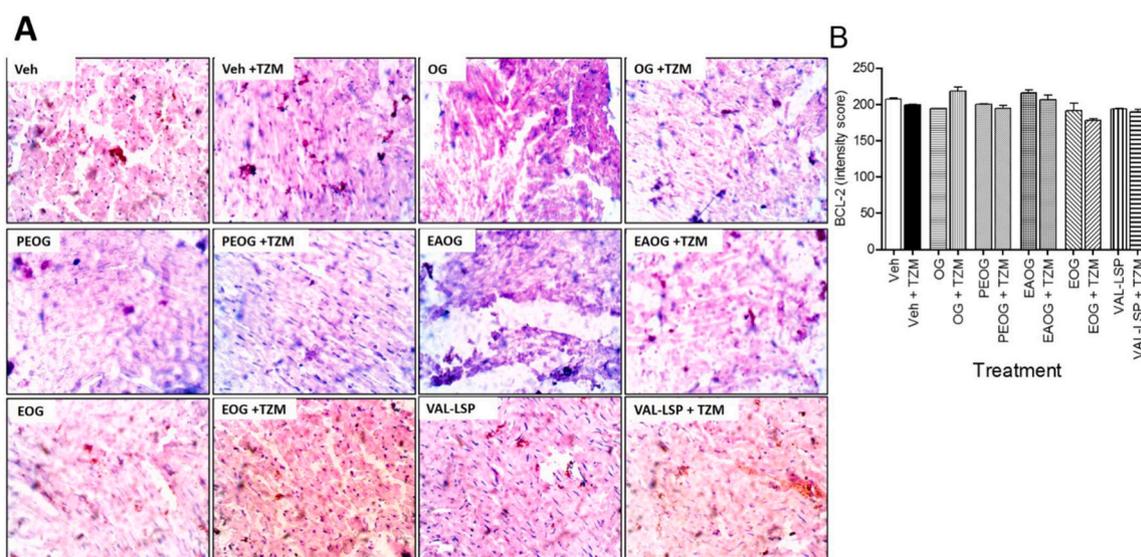

Fig. 7. . (A) Representative section of photomicrographs of immunohistochemical staining of cardiac tissue BCL-2 expression (magnification x 400), and (B) Intensity score of expressions. Bar represents mean $\pm$ SEM (n = 3), significant difference denoted by #p < 0.05 vs Veh.-only treated or *p < 0.05 vs Veh. + *TZM*–treated group by One-way ANOVA followed by Tukey's post hoc test.

this paper.

Data availability

Data will be made available on request.

Author contributions

OEO designed the experimental protocol for this study and was involved in the manuscript writing and editing; AAA also designed, supervised the research, analyzed data and wrote the manuscript; AOA is a postgraduate student in OEO's Laboratory and assisted throughout the experiment; OA was involved in the extraction and solvent partitioning of *OG*; AMA conducted the lipids, antioxidant markers and apoptosis markers assays and was also involved in data analysis; IIO

prepared the cardiac tissue slides for histopathological studies; SSS and AIM independently interpreted the prepared histology slides of the treated cardiac tissues; RMA is our collaborator in the U.S.A. who was involved in the conceptualization, proof-read and edited the manuscript.

6. Contribution to the field statement

Clinical use of *TZM* has been widely limited by its cumulative but reversible off-target cardiotoxicity. In this study therapeutic potential of *OG* and its solvent fractions in mitigating *TZM*-mediated cardiotoxicity is proposed. This theory relies on cardiac injury enzyme markers, cardiac oxidative stress markers, histological examination and immunohistochemical markers as the therapeutic evaluation endpoints of *OG*, *OG* fractions and VAL-LSP fixed dose combination in *TZM*-intoxicated rats.

Results of the study showed that that repeated *TZM* treatment caused remarkable elevations in the plasma LDL-c levels, atherogenicity and coronary artery indices, cardiac troponin I levels, and cardiac tissue caspases-3 and − 9 levels but decreased BCL-2 expression. *TZM* also significantly reduced CAT, SOD, GST and GPx activities and increased MDA levels in the treated cardiac tissues. In addition, *TZM* cardiotoxicity was characterized by severe vascular congestion and cardiomyocyte degeneration and cartilaginous degeneration. Oral pretreatments with *OG*, *OG* fractions (especially *EAOG* fraction) and VAL-LSP fixed dose combination significantly prevented and reversed the biochemical, histopathological and immunohistochemical alterations. Based on our novel findings which we are reporting for the first time, we establish a case for the therapeutic potentials of *OG*, *EAOG* fractions and VAL-LSP fixed dose combination in *TZM*-mediated cardiotoxicity which is probably mediated via anti-apoptosis and antioxidant mechanisms.

Ethics approval

Ethical approval (UERC Approval number: UERC/ASN/2022/2327) for the study was obtained from the University of Ilorin Ethical Review Committee for Staff and Postgraduate Research, University of Ilorin, Kwara State, Nigeria.

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