INTRODUCTION

A number of cytotoxic agents that are metabolized and excreted from the body through the hepatic and renal routes may significantly alter the functional integrity of liver and kidney due to the toxic nature of either their primary or secondary metabolites, although the severity and pattern of their toxicity vary according to their respective drug targets [1]. These anticancer agents include alkylating cytotoxic agents, antibiotic cytotoxics, molecular targeted therapies, radio-diagnostic contrast agents as well as bone targeted therapies depending on the cancer types and stages [1].

Trastuzumab (TZM) is a humanized mouse IgG1 kappa monoclonal antibody targeted against the subdomain IV of the extracellular region of human epidermal growth factor receptor 2 (HER2) [2-4] which is widely used in the clinical management of HER2 overexpressing metastatic solid tumors such as breast and gastric cancers [4,5], adenocarcinoma of gastroesophageal junction [2,3,6], and advanced HER2-positive salivary duct carcinoma and colorectal carcinoma [7]. TZM’s cytotoxic mechanisms are generally believed to be multimodal [3,5] and include: Interference with signal transduction pathways, impairment of extracellular domain cleavage, inhibition of carcinogenesis [8-10], and activation of antibody-dependent cellular cytotoxicity [3,5,8,9]. Despite the huge success already recorded with its clinical use, TZM is notorious for causing cumulative but reversible off-target organ toxicities such as cardiotoxicity [10-12], hepatotoxicity [13,14], and nephrotoxicity [4,15-17].
hematotoxicity [18-22], interstitial pneumonitis [23-26], and infusion-related hypersensitivity reactions [27]. There are reports that prolonged TZM administration is associated with renal dysfunctions [15,28,29] that may manifest as acute kidney injury (AKI) (which itself is characterized by increased serum creatinine, electrolytes imbalance, and impaired glomerular function) [5]. Similarly, TZM has been reported to cause hepatotoxicity which is often characterized by marked elevation in the serum hepatic enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) [9,13,14,30-32]. The dose-dependent hepatotoxicity has been reported to be related to increased hepatic tissue Kupffer cells recruitment and elevated TNF-α gene expression resulting in increased pro-inflammatory cytokines release [31]. Thus, the hallmarks of TZM-induced hepatotoxicity are inflammation and/or necrosis [33]. Unfortunately, there are no known approved antidotes to these off-target toxicities.

*Irvingia gabonensis*, belonging to Irvingiaceae family, is commonly called African/bush/wild mango because of its mango-like fruits and is abundantly distributed in the West African tropical forest [34]. While its fruits are abundantly rich in oil which can be used in the makings of bread and other confectioneries, butter, soap, and livestock feeds, its fruit kernels are rich source of fat, oil, and protein and making it popularly used as soup condiments and thickener [11]. Its sweet pulps are used in the folkloric treatment of diabetes, ulcer, arthritis, rheumatism, and dropsy [48,49]. Erukainure et al. [50] reported the antioxidant, immunomodulatory, and anti-inflammatory properties of protocathechuic acid and dietary fatty acids isolated from *Clerodendrum volubile* leaves against human breast cancer [51] and prostate cancer [52] cell lines. Antihyperglycemic and antihyperlipidemic activities of 5,7,4′-trimethoxykaempferol and 4′-methoxy-5,7-dihydroxy isoflavone (biochanin) isolated from *C. volubile* leaves have also been reported in albino Wistar rats [53]. *C. volubile* leaves have equally been reported to inhibit α-amylase, α-glucosidase, and angiotensin-converting enzyme [54].

Afolabi et al. [55] have previously reported the role of angiotensin converting enzyme inhibitors in the prevention of TZM- and doxorubicin-induced cardiotoxicities. In addition, ranolazine a new anti-ischemia drug and, a specific and potent late sodium current inhibitor, has been reported to attenuate TZM-induced cardiac dysfunction which is known to mediate its action through inhibition of reactive oxygen species (ROS) production [56] and upregulation of antioxidant enzymes [57]. Recently, we reported the protective effect of *C. volubile* ethanol leaf and *I. gabonensis* ethanol seed extract against TZM-induced cardiotoxicity in rats [58]. However, there are no effective antidotes to TZM-mediated hepatorenal toxicities reported so far. In view of this, the present study is the first study designed to evaluate the possible therapeutic potential of *C. volubile* ethanol leaf extract and *I. gabonensis* ethanol seed extract in acute TZM-induced hepatorenal dysfunction in Wistar rats, being the closest phylogenetically to humans.

**MATERIALS AND METHODS**

**Plant materials**

Aerial parts of *C. volubile* and fresh seeds of *Irvingia gabonensis* were purchased from Herbal Vendors in Isikan Market in Akure, Ondo State, Nigeria. Samples of the *C. volubile* plant as well fresh leaves, inflorescence, and fruits of *I. gabonensis* were subjected to botanical identification, authentication, and referencing (voucher specimen number: UY/001/2019/1254 and UY/001/2019/1364, respectively) as previously reported by Akinsola [59].

**Extraction processes**

The extraction process of the fresh leaves of *C. volubile* and pulverized *I. gabonensis* seeds and their % yields calculated as described by Olorundare et al. [58].

**Experimental animals**

After an institutional ethical approval (UERC Approval number: UERC/ASN/2020/2072) was obtained, young adult male Wistar rats (age: 8–12 weeks old) were procured from the Lagos State University College of Medicine Animal House. The rats were processed in accordance with international principles guiding the Use and Handling of Experimental Animals [60]. Rats were generously placed on standard rat chow and potable water and maintained under standard laboratory conditions (ambient temperature: 28–30°C, humidity: 55±5%, and natural photoperiod: 12/12 h alternating light and dark periodicity).

**Body weight measurement**

Rat weights were measured on days 1 and 7 of the experiment and expressed in grams (g).

**Induction of TZM-induced hepatorenal toxicity and other drug treatment of rats**

Random allotment of rats into the different treatment groups and their treatments were as done as previously described by Olorundare et al. [58]. Similarly, choice of the therapeutic doses of 400 mg/kg/day of *C. volubile* ethanol leaf extract (CVE) and *I. gabonensis* ethanol seed extract (IGE) was made based on our previous study [59]. Briefly described, Group I rats were treated with 10 ml/kg/day sterile water p.o. 3 h before 1 ml/kg/day sterile water i.p. injection; Groups II and III rats were orally pretreated with 400 mg/kg/day CVE and IGE, respectively, 3 h before 1 ml/kg/day i.p. sterile water injection; Group IV rats were orally pretreated with 10 ml/kg/day sterile water 3 h before 2.25 mg/kg/day i.p. TZM; and Groups V-VII rats were pretreated with 20 mg/kg/day Vit. C, 400 mg/kg/day CVE and 400 mg/kg/day IGE, respectively, 3 h before i.p. injections of 2.25 mg/kg/day TZM. All treatments were for 7 days.

**Collection of blood samples**

Treated rats were humanely sacrificed under light inhaled diethyl ether anesthesia after an overnight fast. Whole blood samples were obtained directly from the heart with fine 21G needle and 5 ml syringe into plain blood sample bottles.

**Measurement of liver and kidney weights**

Rat livers and kidneys were carefully identified, freed from adjoining supporting tissues, harvested en bloc, and weighed.

**Biochemical assays**

Following blood samples collection, blood samples were allowed to clot at room temperature for 6 h after which they were then centrifuged at 5000 rpm for 15 min to separate out clear sera. Sera obtained were analyzed for the serum liver function parameters (liver enzymes [ALT, AST, and ALP], proteins [TP and ALB], lipids [TG, TC, HDL-c, LDL-c, and VLDL-c] and TB), and renal function parameters (electrolytes [Na, K, Cl and HCO₃⁻], urea, and creatinine) using standard procedures. Serum lipids were assayed using methods of Tietz et al. [61], while serum liver enzyme activities and proteins were estimated using standard bioassay procedures [62].

**Determination of the rat hepatic and renal tissue antioxidant activities**

Following sacrifice of treated rats humanely under light inhalational diethyl ether, liver and kidneys were identified, freed of adjoining connective tissue, dissected out en bloc, and briskly rinsed in normal
saline water. The left and middle lobes of the liver were dissected out carefully with a new surgical blade and the left kidney was briskly rinsed in ice cold 1.15% KCl solution to preserve the oxidative enzyme activities of the liver and kidney before being frozen up on ice packs. Hepatic and renal tissue superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) activities as well as reduced glutathione and malondialdehyde levels were determined as described by Olorundare et al. [58].

**Histopathological evaluation of hepatic and renal tissues**

In conducting histopathological evaluation of livers and kidneys of treated rats, the dissected right lobe of the liver and right kidney of the treated rats were processed for histopathological evaluation using procedures described by Sloufi and Fiette [63]. Prepared thick sections of the processed tissues were that the tissues were subsequently stained with hematoxylin-eosin stain and examined under a photomicroscope coupled with a host computer.

**Statistical analysis**

Data were presented as mean ± S.D. and mean ± S.E.M. of seven samples for the body weight and biochemical parameters, respectively. Data were analyzed using two-way analysis of variance followed by Student-Newman-Keuls test as the post hoc test, on GraphPad Prism Version 5. Statistical significance were considered at p < 0.05, p < 0.01, and p < 0.001.

**RESULTS**

**Extraction process and calculation of %yield**

%yield calculated following the complete extraction of the pulverized *C. volubile* dried leaves was 8.39% with a resultant dark color, sticky and jelly-like, and sweet-smelling solid residue which was completely insoluble in water but completely soluble in methanol and ethanol. Similarly, complete extraction of *I. gabonensis* ethanol seed extract in absolute ethanol resulted in a dark brown, oily, and aromatic residue that was only soluble in methanol and ethanol with a yield of 4.31%.

**Effect of CVE and IGE on the body weight changes and relative organ weights of treated rats**

Table 1 depicts the effects of repeated daily intraperitoneal injection with 2.25 mg/kg of TZM and oral pretreatments with 20 mg/kg/day of Vitamin C and 400 mg/kg/day of CVE and IGE, respectively, on the average body weight on days 1 and 7 and percentage weight change (%Δwt.). TZM treatment had no significant (p > 0.05) effect on the body weight and kidney weights. Similarly, body weights of the CVE-treated rats were significantly (p < 0.05) attenuated on the 7th day compared to the control rats (Group I). However, oral 400 mg/kg/day of CVE and IGE pretreatments resulted in significant (p < 0.05) reduction of body weight in the treated rats (Groups I-IV) (Table 1). However, repeated oral treatments with 400 mg/kg/day of CVE and IGE significantly (p < 0.05 and p < 0.0001) attenuated alterations in the activities of these enzyme markers in the cardiac tissue restoring their activities to normal as recorded for Groups I-III values. These values were also comparable to those of Vit. C-treated rats. However, oral 400 mg/kg/day of CVE and IGE on the renal tissue oxidative stress markers (GSH, GST, GPx, SOD, CAT, and MDA) of TZM-treated rats

**Effect of oral pretreatments of 400 mg/kg/day of CVE and IGE on serum liver function and lipids in TZM-intoxicated rats**

Repeated intraperitoneal treatments with 2.25 mg/kg of TZM resulted in significant (p < 0.001) elevations in the serum liver enzymes (ALT, AST, and ALP) while causing significant (p < 0.0001) reductions in the serum proteins (TP and ALB) but had no effect on the serum TB (Table 2). Similarly, TZM treatment caused no significant (p > 0.05) alterations in the serum lipids (Table 3). However, oral 400 mg/kg/day of CVE and IGE pretreatments resulted in significantly (p < 0.001 and p < 0.0001) attenuated reductions in the serum liver enzymes and proteins. Furthermore, CVE and IGE pretreatments did not significantly (p > 0.05) alter serum levels of TB and lipids (Tables 2 and 3).

**Effect of 400 mg/kg/day of CVE and IGE on the serum renal function in TZM-intoxicated rats**

Repeated intraperitoneal treatments with 2.25 mg/kg of TZM resulted in significant (p < 0.0001) reductions in the serum Na⁺, Cl⁻, and HCO₃⁻ while causing significant (p < 0.001 and p < 0.0001) increases in the serum K⁺, urea, and creatinine (Table 4). With 400 mg/kg/day of CVE and IGE pretreatments, there was significant attenuation (p < 0.0001) in the reductions of the serum Na⁺, Cl⁻, and HCO₃⁻ while elevations in the serum K⁺, urea, and creatinine were significantly attenuated (p < 0.001 and p < 0.0001) (Table 4).

**Effect of CVE and IGE on the hepatic tissue oxidative stress markers (GSH, GST, GPx, SOD, CAT, and MDA) of TZM-treated rats**

Repeated TZM treatments resulted in significant attenuation (p < 0.05 and p < 0.0001) in SOD, CAT, GST activities, and GSH levels while there were significant increases (p < 0.001) in the GPx and MDA activities (Table 5). However, CVE and IGE pretreatments significantly (p < 0.05, p < 0.0001) attenuated alterations in the activities of these enzyme markers in the cardiac tissue restoring their activities to normal as recorded for Groups I-III values. These values were also comparable to those of Vit. C-treated rats (Table 5).

**Effect of CVE and IGE on the renal tissue oxidative stress markers (GSH, GST, GPx, SOD, CAT, and MDA) of TZM-treated rats**

TZM treatment resulted in significant attenuation (p < 0.05 and p < 0.0001) in SOD, CAT, GST activities, and GSH levels while there were significant increases (p < 0.001) in the GPx and MDA activities (Table 6). However, repeated oral treatments with 400 mg/kg/day of CVE and IGE significantly (p < 0.05 and p < 0.0001) attenuated alterations in the activities of these enzyme markers in the treated hepatic and renal tissues restoring their activities to normal as recorded for Groups I-III values. These values were also comparable to those of Vit. C-treated rats (Table 6).

**Histopathological assessment of CVE and IGE on TZM-treated hepatic tissues**

Liver tissues repeatedly injected with 2.25 mg/kg/day of TZM through the intraperitoneal route were characterized by marked dilated hepatic sinusoids with vascular congestion, and marked perportal neutrophilic infiltrations (Fig. 1) while those of Groups I-III showed no remarkable hepatic histarchitectural changes (Figs. 2-4). However, repeated oral

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**Table 1: Effect of repeated oral pretreatments with 400 mg/kg/day of CVE and IGE on the average body weights on days 1 and 7, percentage change in weight (%Δwt.) and relative liver (RLW), and kidney weight (RKW) of TZM-treated rats**

| Group | Day 1 bwt. (g) | Day 7 bwt. (g) | %Δwt. | RLW | RKW |
|-------|---------------|---------------|-------|-----|-----|
| I     | 175.80±25.18  | 183.90±20.45  | 0.51±0.92 | 0.69±0.15 | 0.56±0.05 |
| II    | 178.20±27.90  | 189.90±34.42  | 0.24±0.05 | 0.92±0.10 | 0.60±0.04 |
| III   | 183.40±37.69  | 190.00±39.87  | 0.54±0.92 | 0.09±0.15 | 0.64±0.02 |
| IV    | 177.10±20.37  | 188.50±23.86  | 0.37±0.92 | 0.63±0.10 | 0.72±0.04 |
| V     | 176.20±20.46  | 185.00±23.47  | 0.95±0.43 | 0.35±0.17 | 0.68±0.02 |
| VI    | 171.50±21.40  | 180.70±22.94  | 0.99±0.24 | 0.03±0.15 | 0.74±0.04 |
| VII   | 175.80±25.18  | 183.90±20.45  | 0.51±0.92 | 0.69±0.15 | 0.56±0.05 |

*Represents a significant increase at p < 0.05 when compared to Groups I and II values while *Represents a significant decrease at p < 0.05 when compared to Group IV values.
Table 2: Effect of 400 mg/kg/day of CVE and IGE on serum liver enzymes (ALT, AST, and ALP), proteins (TP and ALB) and total bilirubin (TB) in TZM-treated rats

| Groups | Serum liver function parameters |
|--------|---------------------------------|
|        | ALT (U/L) | AST (U/L) | ALP (U/L) | TP (g/L) | ALB (g/L) | TB (mg/dL) |
| I      | 73.4±9.4  | 40.7±48.9 | 115±16.4 | 74.3±0.1 | 26.3±0.9 | 0.020±0.00 |
| II     | 78.9±9.5  | 41.9±46.3 | 127±21.1 | 73.4±0.1 | 27.3±0.1 | 0.020±0.00 |
| III    | 77.7±8.9  | 40.3±58.8 | 97.6±13.8 | 75.1±0.1 | 28.3±0.1 | 0.020±0.00 |
| IV     | 162±0.09b | 60.6±9.19b | 234±26.3b | 63.6±0.17b | 21.0±0.11b | 0.020±0.00 |
| V      | 125±3±2.7  | 373±7.39b | 173±16.2b | 77.7±0.22b | 29.0±0.11b | 0.020±0.00 |
| VI     | 105±1.22c  | 256±12.07b | 157±17.7b | 85.1±0.18b | 35.3±0.11b | 0.020±0.00 |
| VII    | 95.7±12.66 | 197.7±34.3 | 139±16.1b | 83.9±0.21b | 37.1±0.44b | 0.020±0.00 |

Table 3: Effect of 400 mg/kg/day of CVE and IGE on serum lipid profile of TZM-treated rats

| Groups | Serum lipids |
|--------|--------------|
|        | TG (mmol/l) | TC (mmol/l) | HDL-c (mmol/l) | LDL-c (mmol/l) | VLDL-c (mmol/l) |
| I      | 1.00±0.11   | 3.17±0.11  | 0.40±0.03       | 0.51±0.10       | 0.45±0.05       |
| II     | 0.79±0.06   | 1.4±1.13   | 0.41±0.04       | 0.64±0.08       | 0.36±0.04       |
| III    | 0.79±0.09   | 1.47±0.12  | 0.44±0.04       | 0.67±0.09       | 0.36±0.04       |
| IV     | 0.96±0.05   | 1.53±1.09  | 0.44±0.02       | 0.66±0.09       | 0.43±0.02       |
| V      | 0.94±0.10   | 1.51±1.00  | 0.44±0.02       | 0.64±0.07       | 0.43±0.04       |
| VI     | 0.86±0.09   | 1.4±1.13   | 0.40±0.03       | 0.62±0.07       | 0.39±0.04       |
| VII    | 0.80±0.06   | 1.4±1.13   | 0.42±0.03       | 0.67±0.04       | 0.36±0.03       |

Table 4: Effect of 400 mg/kg/day of CVE and IGE on renal function parameters of TZM-treated rats

| Group | Serum electrolytes, urea, and creatinine |
|-------|-----------------------------------------|
|       | Na+ (mmol/L) | K+ (mmol/L) | Cl- (mmol/L) | HCO3- (mmol/L) | Urea (mmol/L) | Crea (mmol/L) |
| I     | 137.0±0.44  | 6.06±0.30  | 102.0±0.55  | 19.5±0.57      | 9.10±0.16     | 52.10±3.8    |
| II    | 138.3±0.18  | 8.06±0.30  | 104.0±0.22  | 17.29±0.47     | 6.80±0.36     | 54.61±1.25   |
| III   | 139.0±0.57  | 1.49±0.38  | 103.4±0.43  | 17.86±0.59     | 6.85±0.52     | 54.76±1.17   |
| IV    | 125.9±1.20b | 0.93±0.38  | 83.29±2.58b | 10.86±0.96b    | 11.09±0.33b   | 62.04±1.60b  |
| V     | 139.0±0.46b | 0.71±0.53b | 100.4±0.69b | 16.71±0.68b    | 0.74±0.22b    | 62.86±1.12b  |
| VI    | 137.0±0.91b | 0.70±0.70b | 100.3±1.02b | 17.29±1.38b    | 0.72±0.36b    | 57.73±2.53b  |
| VII   | 140.0±0.76b | 0.70±0.63b | 99.5±1.02b  | 18.00±1.33b    | 0.77±0.53b    | 63.26±1.68b  |

Table 5: Antioxidant activities of 400 mg/kg/day of CVE and IGE in TZM-intoxicated rat hepatic tissue

| Groups | Antioxidant parameters |
|--------|------------------------|
|        | GSH (µmol/ml) | GST (µmol/ml) | GPX (µmol/ml) | SOD (µmol/ml/min/mg pro) | CAT (µmol/ml) | MDA (µmol/ml) |
| I      | 1.50±9.48      | 35.55±9.34   | 52.16±5.15   | 0.23±0.14              | 12.78±0.90   | 5.96±0.61   |
| II     | 51.9±1.33      | 37.10±0.64   | 56.92±1.74   | 0.19±0.23              | 14.35±0.95   | 7.15±0.23   |
| III    | 55.0±6.29      | 34.53±0.56   | 60.53±7.22   | 0.91±0.26              | 16.70±0.48   | 6.06±0.93   |
| IV     | 35.79±4.47     | 25.4±0.77    | 79.56±3.67   | 0.18±0.04              | 0.16±0.25    | 16.36±0.60  |
| V      | 60.3±1.88      | 35.2±0.67b   | 43.99±0.67   | 0.20±0.04              | 16.08±1.17b  | 0.44±0.30b  |
| VI     | 61.0±1.25      | 37.88±1.39b  | 49.47±1.12   | 0.27±0.20              | 15.49±0.98b  | 0.14±0.04b  |
| VII    | 60.3±1.62      | 54.95±4.00   | 41.63±2.78   | 0.26±0.11              | 13.43±0.18b  | 0.83±0.05b  |

 Pretreatments with 400 mg/kg/day of CVE (Fig. 5) and 400 mg/kg/day of IGE (Fig. 6) significantly improved these TZM-induced hepatic lesions while oral pretreatment with 20 mg/kg/day of Vit. C showed no remarkable improvements in the hepatic lesions in the TZM-intoxicated rats (Fig. 7).
While those of Groups I-III were of no remarkable renal histoarchitectural changes that were orally treated with 10 ml/kg/day of sterile water, 400 mg/kg/day of CVE, and 400 mg/kg/day of IGE only, respectively (Figs. 9-11). However, these marked histological lesions induced by TZM were markedly improved by repeated oral pretreatments with 400 mg/kg/day of CVE (Fig. 12) and 400 mg/kg/day of IGE.

### Table 6: Antioxidant activities of 400 mg/kg/day of CVE and IGE in TZM-intoxicated rat kidney tissue

| Groups | GSH (µmol/ml) | GST (µmol/ml) | GPx (µmol/ml) | SOD (µmol/ml/min/mg pro) | CAT (µmol/ml) | MDA (µmol/ml) |
|--------|---------------|---------------|---------------|--------------------------|---------------|---------------|
| I      | 78.5±4.06     | 37.2±1.11     | 68.7±2.34     | 04.72±0.37               | 19.6±1.00     | 01.06±0.20    |
| II     | 61.8±6.17     | 38.6±1.71     | 63.3±3.48     | 03.97±0.27               | 19.48±1.92    | 01.01±0.20    |
| III    | 56.7±5.84     | 34.6±0.47     | 59.2±5.24     | 04.06±0.18               | 21.84±1.98    | 00.91±0.20    |
| IV     | 27.1±1.56     | 24.9±1.27     | 83.9±2.65     | 02.83±0.27               | 09.93±1.10    | 09.25±0.81    |
| V      | 53.5±6.89     | 37.5±0.90     | 52.6±0.74     | 05.79±0.84               | 12.99±0.70    | 00.69±0.21    |
| VI     | 50.2±2.90     | 34.2±0.28     | 34.0±2.66     | 05.28±0.57               | 15.67±1.00    | 00.77±0.20    |
| VII    | 52.4±3.10     | 38.9±1.37     | 24.0±3.15     | 18.37±0.80               | 12.67±0.13    | 00.42±0.07    |

*Represents a significant decreases at p<0.0001 when compared to Groups I-III (controls) values while † and ‡ Represent significant increases at p<0.05 and p<0.0001, respectively, when compared to Group IV values; ‡ Represents a significant increase at p<0.05 when compared to Groups IV values while † and ‡ Represent significant decreases at p<0.05 and p<0.0001, respectively, when compared to untreated positive control (TZM treated only, Group IV). CVE: Clerodendrum volubile ethanol leaf extract, IGE: Irvingia gabonensis ethanol seed extract, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GST: Glutathione-S-transferase
of IGE (Fig. 13) with 20 mg/kg/day of Vit. C showing no remarkable improvements in the TZM-induced renal vascular congestion, and hyaline arteriosclerosis (Fig. 14).

DISCUSSION

Herbal remedy as an important therapeutic approach either alone or in combination with the well-established orthodox medicines is a viable tool for the provision of adequate and robust health care [64-66]. Identifiable factors encouraging inclusion of herbal regimen in healthcare management in both developed and developing countries include affordability, easy availability, and accessibility [67,68]. In addition, the field of traditional, complementary, and alternative medicines supports the use of phytomedicinal plants and nutraceuticals as therapeutic/chemopreventive agents and for use in combating resistance and ameliorating the toxic side effects several chemotherapeutic agents, due to scientific reports confirming their efficacy in preclinical and clinical models [69].

In the present study, chemotherapeutic potentials of 400 mg/kg/day of CVE and IGE were investigated in TZM-mediated hepatorenal toxicities using measuring outcome endpoints such as the hepatic function parameters, renal function parameters, oxidative stress markers, and histopathological endpoints.

TZM-induced hepatotoxicity is reported to be characterized by marked dose-related elevations in the serum liver enzyme markers such as ALT, AST, and ALP [13,30,32,70]. ALT and AST are found within the hepatocytes and are only released when there is liver damage although the cardiac muscles equally contain certain quantity of AST, while ALP is found in the cell lining of the hepatic biliary duct and released in large amount when there is hepatic biliary duct injury or obstruction [71-74]. ALP may also be profoundly elevated in metastatic hepatic carcinoma and metastatic colon carcinoma [75,76], lymphoma [77-82], osteosarcoma [83,84], or infiltrative diseases
such as sarcoidosis [85-87]. Thus, elevations in circulating ALT and AST levels are regarded as reliable markers of intrahepatic injury, including acute hepatotoxicity while elevations in the circulating ALP is indicative of extrahepatic biliary injury, which is more often extrahepatic cholestatic obstruction [88,89]. The fact that these liver enzyme markers were markedly elevated following repeated TZM injections in this study is indicative that TZM-induced hepatotoxicity was fully established. Oral pretreatments with 400 mg/kg/day of CVE and IGE profoundly attenuated increases in the serum levels of these enzyme markers and reflecting their protective activities against TZM-mediated hepatotoxicity. Apart from liver enzymes, other biochemical parameters, such as the serum proteins (TP and ALB), lipids, and TB are considered corollary biomarkers of liver functions and their alterations are known to provide insight into liver function integrity [90-92]. In liver diseases including drug-induced hepatotoxicity, the circulating levels of these corollary liver function parameters which are synthesized de novo in the liver are decreased except for TB that may either be elevated or unaffected depending on whether the cause of the liver disease/injury is pre-hepatic, hepatic, or post-hepatic [93-95]. Aside liver enzymes, TZM is also known to induce profound elevations in the serum total bilirubin, prothrombin time/international normalized ratio [13], decrease serum ALB and LDH levels [96], and serum lipids and other metabolomics profile [97]. Contrary to other reports, TZM, in this study, caused unremarkable alterations in the serum TB and serum lipid levels. However, the fact that CVE and IGE oral pretreatments profoundly attenuated decreases in the serum protein levels strongly lends support to the protective activity of these extracts against TZM-induced hepatotoxicity. These remarkable alterations in the hepatic enzymes and other hepatic function parameters were corroborated by hepatic histopathological lesions of dilated sinusoidal congestion and neutrophilic infiltration which were improved remarkably by CVE and IGE oral pretreatments.

TZM is also known to mediate deleterious effects of the renal function which may manifest as AKI and electrolyte imbalance [5,98,99]. AKI may manifest as profound increases in the serum creatinine and urea, hypomagnesemia, hypokalemia, hypophosphatemia, hypocalcemia [5,100], as well as metabolic acidosis [101]. In this study,
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Oxidative and nitrosative stress have been implicated in the biopathology of TZM-mediated hepatorenal toxicities through generation of highly toxic ROS and nitrosative species by interfering with HER-2 signaling and inhibiting tissue pro-survival effects [30,103-105]. TZM is known to interfere with mitochondrial functionality and causing mitochondrial dysfunction, ATP depletion and inhibiting AMPK and PI3K/Akt pathways [105,106]. TZM activates proapoptotic pathway proteins such as Bax and can induce the opening of mPTP, consequently resulting in mitochondrial dysfunction and ROS accumulation [107]. Similarly, TZM also binds to HER-2 and increases proapoptotic Bcl-xS expression while it decreases antiapoptotic Bcl-2 expression [108]. These results in overwhelming ROS production and reduced ROS scavenging activities leading to markedly reduced SOD, CAT GST activities, and GSH levels and enhanced GPx activities and MDA levels in TZM-treated tissues [109] which results of this present study are strongly in line with. However, with CVE and IGE pretreatments, there were profound improvements in the oxidative stress markers in both TZM-treated hepatic and renal tissues, indicating the protective role of CVE and IGE in TZM-induced tissue oxidative stress.

Secondary metabolites such as terpenoids, alkaloids, and polyphenols (including stilbenes, phenolic acids, coumarins, flavonoids, anthraquinones, and tannins) have been documented to elicit powerful free radicals scavenging and antioxidant activities [110]. CVE and IGE have been reported to be rich sources of flavonoids (quercetin and kaempferol), ellagic acid, mono-, di-, and tri-O-methyl-ellagic acid, and their glycosides which are potent antioxidants [49,58,111-113]. The presence of these secondary metabolites in CVE and IGE which previously have been reported to elicit antioxidant activities [47,49,58], including TZM-induced cardiotoxicity [113], was responsible for the significant free radical scavenging and antioxidant activities recorded for CVE and IGE in this study.

CONCLUSION

Overall, findings of this study highlight the promising therapeutic potential of CVE and IGE against TZM-induced hepatorenal dysfunction, partly mediated through hepatic and renal oxidative stress inhibition.

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AUTHORS’ CONTRIBUTION

Olufunke Olorund are designed the experimental protocol for this study and was involved in the manuscript writing; Adejowo Adeneye also designed, supervised the research, analyzed data, and wrote the manuscript; Akinyele Akinsola is an M.Sc. student in Olufunke Olorundare’s laboratory who performed the laboratory research; Sunday Soyemi and Alban Mgbehoma independently evaluated and performed the laboratory research; and Olorundare’s laboratory who performed the laboratory research; in assaying for the serum cardiac biomarkers and lipid profile.

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Fig. 13: A cross-sectional representative of the trastuzumab-intoxicated rat kidney orally pretreated with 400 mg/kg/day/p.o. route of Clerodendrum volubile ethanol leaf extract dissolved in distilled water showing renal vascular congestion (indicated by the blue arrow) and hyaline arteriosclerosis with no interstitial neutrophilic infiltration (×400, Hematoxylin-Eosin stain)

Fig. 14: A cross-sectional representative of the trastuzumab-intoxicated rat kidney orally pretreated with 20 mg/kg/day/p.o. route of Vit. C dissolved in distilled water showing renal vascular congestion (indicated by the blue arrow), hyaline arteriosclerosis with moderate interstitial neutrophilic infiltration (indicated by the red arrow) (×400, Hematoxylin-Eosin stain)
CONFLICTS OF INTEREST
The authors have none to declare.

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