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Research Article

EXPERIMENTAL THERAPEUTIC EVALUATION OF THE AFRICAN VEGETABLES (*CLERODENDRUM VOLUBILE LEAF* AND *IRVINGIA GABONENSIS* SEED EXTRACTS) IN TRASTUZUMAB-MEDIATED HEPATO-RENAL DYSFUNCTION IN WISTAR RATS

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ABSTRACT

Objective: The use of trastuzumab (*TZM*) in the clinical management of human epidermal growth factor receptor 2 positive metastatic breast and gastric cancers, gastro-esophageal adenocarcinoma, and colorectal carcinoma has been limited by its off-target cardiac, hepatic, and renal toxicities which till date have no effective therapies in either their prevention or amelioration. Thus, the present study is designed at investigating the protective and therapeutic potentials of 400 mg/kg/day *Clerodendrum volubile* ethanol leaf extract (*CVE*) and *Irvingia gabonensis* ethanol seed extract (*IGE*) pretreatments in *TZM*-intoxicated Wistar rats based on their reported folkloric use in the local management of kidney and liver diseases and the previously reported therapeutic potential of these African vegetables in *TZM* cardiotoxicity.

Methods: Forty-nine male Wistar rats were randomly allotted into seven groups of seven rats per group. Group I rats were treated with 10 ml/kg/day of 5% dimethyl sulfoxide (DMSO) sterile water *p.o.* and 1 ml/kg/day 5% DMSO sterile water *i.p.*; 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.* 5% DMSO sterile water; Group IV rats were orally pretreated with 10 ml/kg/day 5% DMSO 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* all dissolved in 5% DMSO sterile water, respectively, 3 h before *i.p.* injections of 2.25 mg/kg/day *TZM*, all for 7 days. Liver function parameters, renal function parameters, oxidative stress markers, and histopathological investigations were the study measuring endpoints.

Results: Oral pretreatment with 20 mg/kg/day Vit. C, 400 mg/kg/day *CVE* and *IGE* significantly ameliorated *TZM*-mediated hepatic and renal toxicities by effectively lowering the serum alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine, and urea levels. *CVE* and *IGE* pretreatments also significantly reversed *TZM*-induced decreases in the hepatic and renal tissue catalase, superoxide dismutase, and glutathione-*S*-transferase activities and reduced malondialdehyde levels. *CVE* and *IGE* pretreatments also improved *TZM*-induced hepatic and renal histological lesions.

Conclusions: Overall, the chemotherapeutic/chemopreventive potentials of *CVE* and *IGE* in *TZM*-induced hepatorenal dysfunction were either wholly or partly mediated through free-radical scavenging and antioxidant activities.

Keywords: Trastuzumab hepatorenal toxicity, Liver and renal function parameters, Oxidative stress markers, *Clerodendrum volubile* ethanol leaf extract, *Irvingia gabonensis* ethanol seed extract, Male Wistar rats.

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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 IgG 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 DNA repair, decreased angiogenesis, induction of cell cycle arrest, 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], 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 it 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 also used in the beverage and winery industries [35]. Different part extracts of the plant have been reported to have various pharmacological values which include anti-obesity and antihyperlipidemic [36-41], antihyperglycemic [42,43], analgesic [44,45], antimicrobial [46], and antioxidant [47] properties.

Clerodendrum volubile, popularly called the "magic leaf" due to its vast array of medicinal uses, is among the common food ingredients in Southern Nigeria where it is locally known as "*Marugbo*" among the Ondo and Ilaje people (Southwest Nigeria) and "*Obenetete*" among the Ijaws (Southern Nigeria). In these areas, decoctions made from *C. volubile* are used in the folkloric treatment of diabetes, ulcer, arthritis, rheumatism, and dropsy [48,49]. Erukainure *et al*. [50] reported the antioxidant, immunomodulatory, and antiproliferative activities of protocatechuic acid and dietary fatty acids isolated from *C. 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 be reported in albino Wistar rat [53]. *C. volubile* leaves have equally been reported to inhibit α-amylase, α-glucosidase, and angiotensin-converting enzyme [54].

Akolkar *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 extracts 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: UIL/001/2019/1254 and UIL/001/2019/1364, respectively) as previously reported by Akinsola [59].

Extraction processes

The extraction processes 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 HCO_3^-], 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 malonialdehyde 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 Slaoui 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 weight gain pattern and relative liver and kidney weights. Same results were recorded for *CVE*-pretreated (Group II), *IGE*-pretreated (Group III), and vit. C-pretreated (Group V) (Table 1). However, *TZM* treatment caused a significant ($p<0.05$) increase in RLW and RKW relative to normal control (Groups I) and *CVE*-only pretreated (Group II) values (Table 1) while there were profound ($p<0.05$) decreases in the RLW and RKW values with *CVE* and *IGE* pretreatments relative to *TZM*-intoxicated group (Group IV) (Table 1). However, Vit. C pretreatment caused no significant ($p>0.05$) changes in the RLW and RKW values when compared to *TZM*-treated values (Table 1).

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 *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 ($p>0.05$) 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 HCO_3^- 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 HCO_3^- 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 Vit. C-treated (Group V) values (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 group (Table 6).

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

Rat livers 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 periportal neutrophilic infiltrations (Fig. 1) while those of Groups I-III showed no remarkable hepatic histoarchitectural changes (Figs. 2-4). However, repeated oral

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	05.12±04.92	02.69 ± 0.15	00.56±0.05
II	178.20±27.90	189.90±34.42	06.24±05.05	02.92 ± 0.10	00.60±0.04
III	183.40±37.69	190.00±39.87	03.54±02.93	03.09 ± 0.15	00.64±0.02
IV	177.10±20.37	188.50±23.61	06.37±02.60	03.36 ± 0.10[a]	00.72±0.04[a]
V	176.20±20.46	185.00±23.47	05.95±05.43	03.25 ± 0.17[a]	00.68±0.02
VI	171.50±17.73	178.40±17.17	04.15±04.11	03.16 ± 0.08[a]	00.74±0.04[a]
VII	171.50±21.40	180.70±22.94	03.99±03.24	03.01 ± 0.15[a]	00.66±0.02[a]

[a]Represents a significant increase at $p<0.05$ when compared to Groups I and II values while [a-]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±04.7	400.7±48.9	115.4±16.4	74.3±02.1	26.3±0.9	00.20±0.00
II	78.9±09.5	419.9±46.3	127.1±21.1	73.4±01.4	27.3±01.3	00.20±0.00
III	77.7±08.9	403.3±58.8	97.6±13.8	75.1±01.5	28.3±01.4	00.20±0.00
IV	162.1±08.9^{b}	606.0±91.9^{b}	231.4±26.3^{b}	63.6±01.7^{f}	21.0±01.1^{f}	00.20±0.00
V	125.0±32.2^{a-}	373.7±84.9^{a-}	173.1±20.6^{a-}	77.7±02.2^{e+}	29.0±01.1^{e+}	00.20±0.00
VI	105.9±12.2^{c-}	256.1±20.7^{b-}	157.9±17.7^{a-}	83.1±01.8^{f+}	35.3±01.1^{f+}	00.20±0.00
VII	95.7±12.6^{c-}	197.7±34.3^{c-}	139.9±16.1^{b-}	83.9±02.1^{f+}	37.1±01.44^{f+}	00.20±0.00

^{b}Represents a significant increase at p<0.001 when compared to Group I-III values while $^{a-}$and $^{b-}$Represent significant decreases at p<0.05 and p<0.001, respectively, when compared to Group IV values; ^{f}Represents a significant decrease at p<0.0001 when compared to controls (Groups I-III) values while $^{e+}$ and $^{f+}$ represent significant increases at p<0.001 and p<0.0001, respectively, when compared to Group IV values. CVE: *Clerodendrum volubile* ethanol leaf extract, IGE: *Irvingia gabonensis* ethanol seed extract, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

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)	VLDC-c (mmol/l)
I	1.00±0.11	1.37±0.11	0.40±0.03	0.51±0.10	0.45±0.05
II	0.79±0.06	1.41±0.13	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±0.09	0.44±0.02	0.66±0.09	0.43±0.02
V	0.94±0.10	1.51±0.10	0.44±0.02	0.64±0.07	0.43±0.04
VI	0.86±0.09	1.40±0.13	0.40±0.03	0.62±0.07	0.39±0.04
VII	0.80±0.06	1.45±0.08	0.42±0.03	0.67±0.04	0.36±0.03

CVE: *Clerodendrum volubile* ethanol leaf extract, IGE: *Irvingia gabonensis* ethanol seed extract

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)	HCO_3^{-} (mmol/L)	Urea (mmol/L)	Crea (mmol/L)
I	137.00±0.44	06.00±0.30	102.10±0.55	19.57±0.57	06.79±0.16	52.10±1.37
II	138.30±0.18	06.30±0.38	104.00±0.22	17.29±0.47	06.80±0.36	54.61±1.25
III	139.40±0.57	06.97±0.38	103.40±0.43	17.86±0.59	06.60±0.52	54.76±1.71
IV	125.90±1.20^{f}	09.13±0.38^{c}	83.29±2.58^{f}	10.86±0.96^{f}	11.09±0.33^{c}	82.04±1.60^{c}
V	139.90±0.46^{f+}	07.51±0.53^{c-}	100.40±0.69^{f+}	16.71±0.68^{f+}	07.74±0.22^{c-}	62.86±1.12^{c}
VI	137.90±0.91^{f+}	07.40±0.70^{c-}	100.30±1.02^{f+}	17.29±1.38^{f+}	07.29±0.36^{c-}	57.73±2.53^{c}
VII	140.00±0.76^{f+}	06.90±0.63^{b-}	99.57±1.02^{f+}	18.00±1.33^{f+}	07.77±0.53^{c-}	63.26±1.68^{c}

^{c}Represents a significant increase at p<0.0001 when compared to Groups I-III values while ^{b}and ^{c}Represent significant decreases at p<0.001 and p<0.0001 when compared to Group IV values; ^{f}Represents a significant decrease at p<0.0001 when compared to Groups I-III values while $^{f+}$Represent a significant increase at p<0.0001 when compared to Group IV values. CVE: *Clerodendrum volubile* ethanol leaf extract, IGE: *Irvingia gabonensis* ethanol seed extract

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	50.99±4.89	35.55±0.34	52.16±5.15	02.37±0.14	12.78±0.90	05.96±0.61
II	51.93±1.33	37.10±0.64	56.92±1.74	01.93±0.23	14.35±0.95	07.15±0.23
III	55.02±6.29	34.53±0.56	60.53±7.22	01.98±0.26	16.70±0.48	06.00±0.93
IV	35.79±4.47^{f}	25.46±0.77^{f}	79.56±3.67^{c}	01.08±0.04^{a}	06.17±0.45^{f}	16.36±0.60^{c}
V	60.30±1.88^{f+}	35.23±0.67^{d+}	43.99±0.67^{c-}	02.08±0.04^{b+}	16.08±1.17^{f+}	04.41±0.30^{c-}
VI	61.01±2.15^{f+}	37.88±1.39^{d+}	49.47±1.12^{c-}	02.76±0.20^{c+}	15.49±0.98^{f+}	01.04±0.07^{c-}
VII	60.36±1.22^{f+}	54.95±4.00^{f+}	41.63±2.78^{c-}	02.69±0.11^{c+}	13.43±0.18^{f+}	0.83±0.05^{c-}

^{f}Represents a significant decrease at p<0.0001 when compared to Groups I-III (controls) values while $^{f+}$Represents a significant increases at p<0.0001 when compared to Group IV values; ^{a} and ^{c}Represent significant increases at p<0.05 and p<0.0001, respectively, when compared to Groups I-III values while $^{b+}$ and $^{c+}$Represents significant decreases at p<0.001 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

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 *TZM*-induced hepatic lesions (Fig. 7).

Histopathological effect of *CVE* and *IGE* on *TZM*-treated renal tissue

Rat kidneys repeatedly injected with 2.25 mg/kg/day of *TZM* through the intraperitoneal route were characterized by marked vascular congestion, hyaline arteriosclerosis with focal neutrophilic infiltrations

Table 6: Antioxidant activities of 400 mg/kg/day of *CVE* and *IGE* in *TZM*-intoxicated rat kidney 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	78.50±4.06	37.29±1.11	68.76±2.34	04.72±0.37	19.64±1.00	01.06±0.20
II	61.85±6.17	38.65±1.71	63.36±3.48	03.97±0.27	19.48±1.92	01.01±0.20
III	56.78±5.84	34.61±0.47	59.29±5.24	04.06±0.18	21.84±1.98	00.91±0.20
IV	27.15±1.56^{f}	24.98±1.27^{f}	83.92±2.65^{c}	02.83±0.27^{f}	09.93±1.10^{f}	09.25±0.81^{c}
V	53.56±5.89^{f+}	37.50±0.98^{f+}	52.60±0.74^{a-}	05.79±0.84^{d+}	12.99±0.70^{e+}	00.69±0.21^{b-}
VI	50.21±2.90^{f+}	34.29±0.28^{f+}	34.01±2.66^{b-}	05.28±0.57^{d+}	15.67±1.00^{f+}	00.77±0.20^{b-}
VII	52.43±2.10^{f+}	38.95±1.37^{f+}	24.01±2.15^{c-}	18.37±0.80^{f+}	12.67±0.13^{e+}	00.42±0.07^{c-}

^{f}Represents a significant decreases at p<0.0001 when compared to Groups I-III (controls) values while $^{d+}$ and $^{f+}$Represent significant increases at p<0.05 and p<0.0001, respectively, when compared to Group IV values; ^{c}Represents a significant increase at p<0.0001 when compared to Groups I-III values while $^{b-}$ and $^{c-}$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

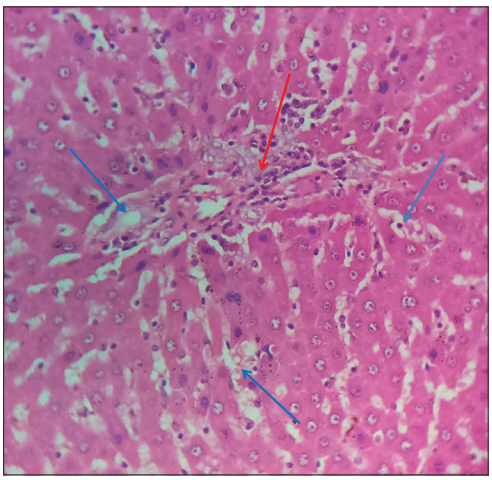

Fig. 1: A cross-sectional representative of the trastuzumab (*TZM*)-intoxicated rat liver treated with 2.25 mg/kg/day/*i.p.* route of *TZM* dissolved in sterile water and pretreated with 10 ml/kg/day/*p.o.* of dissolved in 5% dimethyl sulfoxide sterile water showing dilated hepatic sinusoids, vascular congestion (indicated by the blue arrow), and interstitial neutrophilic infiltration (indicated by the red arrow) (×400, Hematoxylin-Eosin stain)

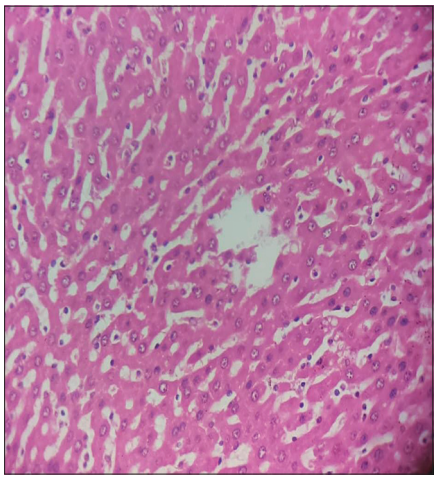

Fig. 2: A cross-sectional representative of the normal rat liver treated with 10 ml/kg/day/*p.o.* route of dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal hepatic histoarchitecture (×400, Hematoxylin-Eosin stain)

(Fig. 8) while those of Groups I-III were of no remarkable renal histoarchitectural changes that were orally treated with 10 ml/kg/day

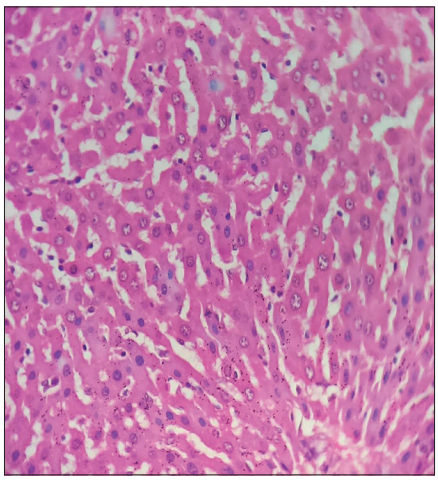

Fig. 3: A cross-sectional representative of the rat liver treated with 400 mg/kg/day/*p.o.* route of *Clerodendrum volubile* ethanol leaf extract dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal hepatic histoarchitecture (×400 magnification, Hematoxylin-Eosin stain)

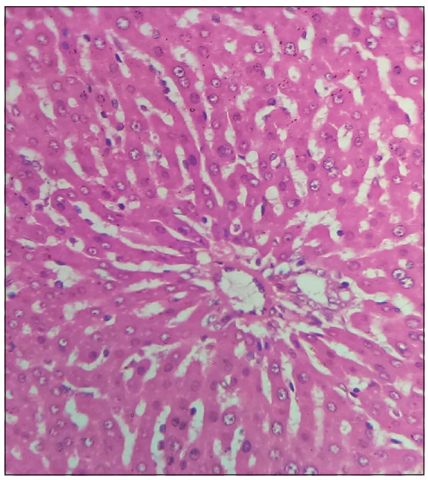

Fig. 4: A cross-sectional representative of the rat liver treated with 400 mg/kg/day/*p.o.* route of *Irvingia gabonensis ethanol seed extract* dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal hepatic histo-architecture (×400, Hematoxylin-Eosin stain)

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

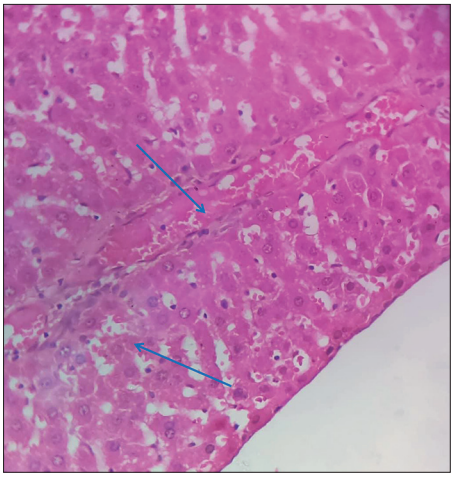

Fig. 5: A cross-sectional representative of the trastuzumab-intoxicated rat liver orally pretreated with 400 mg/kg/day/*p.o.* route of *Clerodendrum volubile* ethanol leaf extract dissolved in 5% dimethyl sulfoxide sterile water showing moderate hepatic vascular congestion and sinusoidal dilatation (indicated by the blue arrow) (×400, Hematoxylin-Eosin stain)

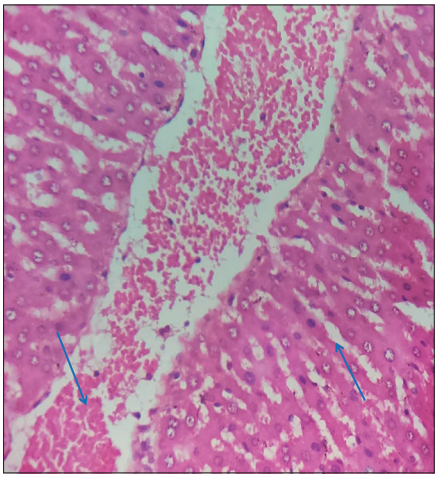

Fig. 6: A cross-sectional representative of the trastuzumab-intoxicated rat liver orally pretreated with 400 mg/kg/day/*p.o.* route of *Irvingia gabonensis ethanol seed extract* dissolved in 5% dimethyl sulfoxide sterile water showing mild hepatic vascular congestion and sinusoidal dilatation (indicated by the blue arrow) (×400, Hematoxylin-Eosin stain)

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 health-care 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].

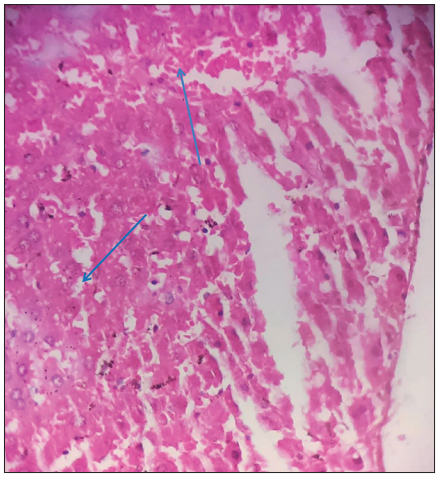

Fig. 7: A cross-sectional representative of the trastuzumab -intoxicated rat liver orally pretreated with 20 mg/kg/day/*p.o.* route of Vit. C dissolved in dissolved in 5% dimethyl sulfoxide sterile water showing dilated hepatic sinusoids and hepatic vascular congestion (indicated by the blue arrows) (×400, Hematoxylin-Eosin stain)

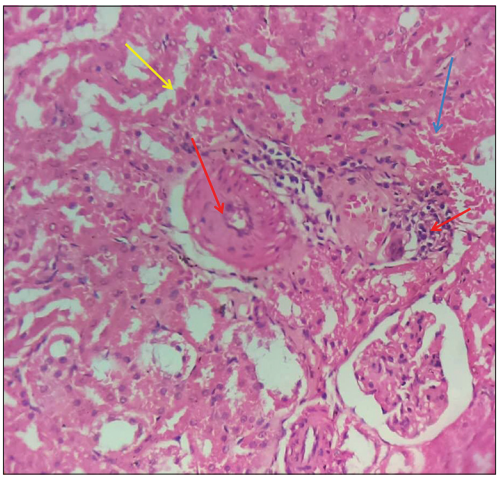

Fig. 8: A cross-sectional representative of the trastuzumab (*TZM*)-intoxicated rat kidney treated with 2.25 mg/kg/day/*i.p.* route of *TZM* dissolved in sterile water and pretreated with 10 ml/kg/day/*p.o.* of dissolved in 5% dimethyl sulfoxide sterile water showing renal vascular congestion (indicated by the blue arrow), hyaline arteriosclerosis with focal neutrophilic infiltration (indicated by the red arrows) and acute tubulointerstitial nephritis (indicated by the yellow arrow) (×400, Hematoxylin-Eosin stain)

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

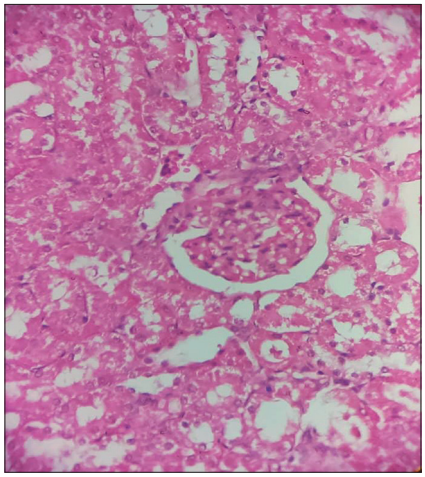

Fig. 9: A cross-sectional representative of the normal rat kidney treated with 10 ml/kg/day/*p.o.* route of dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal renal histoarchitecture (×400, Hematoxylin-Eosin stain)

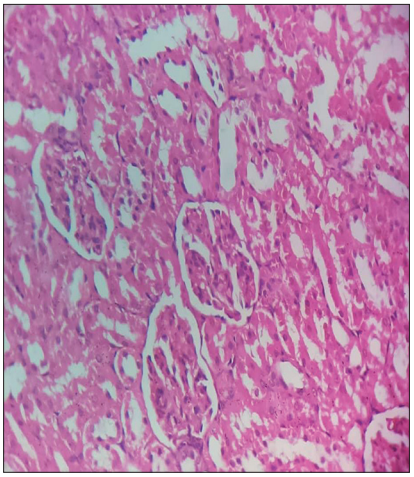

Fig. 11: A cross-sectional representative of the rat kidney treated with 400 mg/kg/day/*p.o.* route of *Irvingia gabonensis ethanol seed extract* dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal renal histoarchitecture (×400, Hematoxylin-Eosin stain)

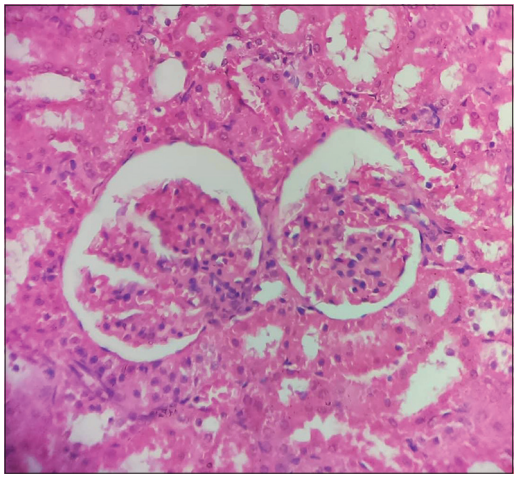

Fig. 10: A cross-sectional representative of the rat kidney treated with 400 mg/kg/day/*p.o.* route of *Clerodendrum volubile* ethanol leaf extract dissolved in 5% dimethyl sulfoxide sterile water and 1 ml/kg/day/*i.p.* route of sterile water showing normal renal histoarchitecture (×400, Hematoxylin-Eosin stain)

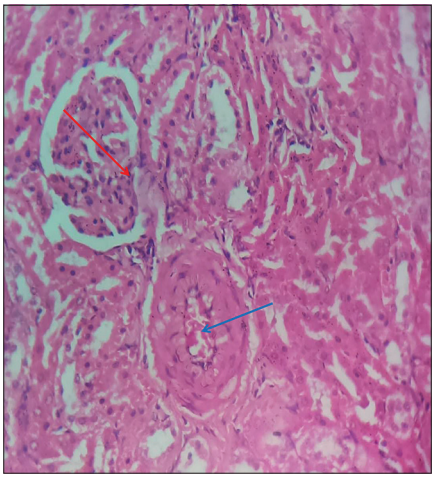

Fig. 12: A cross-sectional representative of the trastuzumab-intoxicated rat kidney orally pretreated with 400 mg/kg/day/*p.o.* route of *Clerodendrum volubile* ethanol leaf extract dissolved in dissolved in 5% dimethyl sulfoxide sterile water showing renal vascular congestion (blue arrow), hyaline arteriosclerosis with mild interstitial neutrophilic infiltration (indicated by the red arrow) (×400, Hematoxylin-Eosin stain)

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 infiltrations 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,

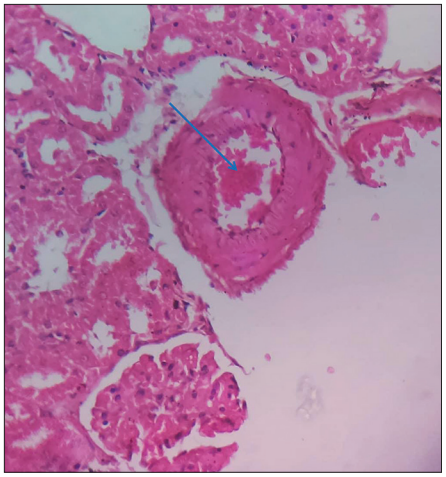

Fig. 13: A cross-sectional representative of the trastuzumab-intoxicated rat kidney orally pretreated with 400 mg/kg/day/*p.o.* route of *Clerodendrum volubile* ethanol leaf extract dissolved in dissolved in 5% dimethyl sulfoxide sterile water showing very mild vascular congestion (indicated by the blue arrow) and hyaline arteriosclerosis with no interstitial neutrophilic infiltration (×400, Hematoxylin-Eosin stain)

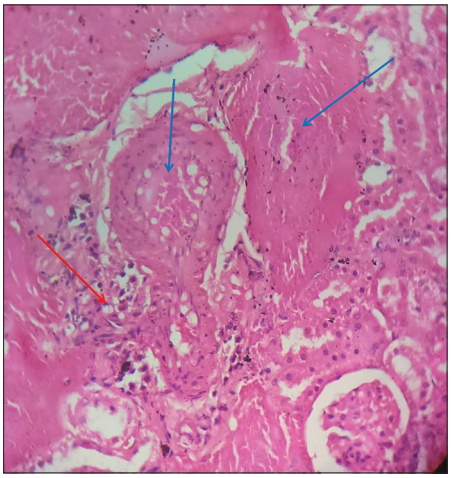

Fig. 14: A cross-sectional representative of the trastuzumab-intoxicated rat kidney orally pretreated with 20 mg/kg/day/*p.o.* route of Vit. C dissolved in dissolved in 5% dimethyl sulfoxide sterile water showing renal vascular congestion (indicated by the blue arrow), hyaline arteriosclerosis with moderate interstitial neutrophilic infiltration (indicated by the red arrow) (×400, Hematoxylin-Eosin stain)

acute *TZM* treatment was associated with hyponatremia, hypochloremia, metabolic acidosis, hyperuremia, and hypercreatininemia which are in line with previous studies [5,101-103]. *TZM* is reported to cause hypokalemia, but hyperkalemia was observed in the current study. This variance could probably be due to acute and/or chronic oliguric kidney which often precedes long-term onset of *TZM*-related hypokalemia [104]. However, these abnormal alterations in the serum electrolytes, urea, and creatinine induced by *TZM* treatment were profoundly improved with *CVE* and *IGE* pretreatments indicating the potential benefits of these extracts in *TZM*-mediated renal dysfunction. Apart from *TZM*-mediated renal function parameter alterations, *TZM* also induced marked vascular congestion, hyaline arteriosclerosis with focal neutrophilic infiltrations, and acute tubulointerstitial nephritis which is similar to that previously reported [17]. However, these *TZM*-induced hepatic histological lesions were remarkably improved by *CVE* and *IGE* oral pretreatments.

Oxidative and nitrative stress have been implicated in the biopathology of *TZM*-mediated hepatorenal toxicities through generation of highly toxic ROS and nitrative 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-xL* 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; Adejuwon 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 reported the prepared histological slides; Ikechukwu Okoye is the Laboratory Technologist who prepared the histopathology slides; Ralph Albrecht, James Ntambi, and Hasan Mukhtar are our collaborators in the U.S.A. who read through the manuscript. In addition, all authors have read and approved the manuscript.

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CONFLICTS OF INTEREST

The authors have none to declare

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