Research Article

Irvingia gabonensis Seed Extract: An Effective Attenuator of Doxorubicin-Mediated Cardiotoxicity in Wistar Rats

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Cardiotoxicity as an off-target effect of doxorubicin therapy is a major limiting factor for its clinical use as a choice cytotoxic agent. Seeds of Irvingia gabonensis have been reported to possess both nutritional and medicinal values which include antidiabetic, weight losing, antihyperlipidemic, and antioxidative effects. Protective effects of Irvingia gabonensis ethanol seed extract (IGESE) was investigated in doxorubicin (DOX)-mediated cardiotoxicity induced with single intraperitoneal injection of 15 mg/kg of DOX following the oral pretreatments of Wistar rats with 100-400 mg/kg/day of IGESE for 10 days, using serum cardiac enzyme markers (cardiac troponin I (cTnI) and lactate dehydrogenase (LDH)), cardiac tissue oxidative stress markers (catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px), and reduced glutathione (GSH)), and cardiac histopathology endpoints. In addition, both qualitative and quantitative analyses to determine IGESE’s secondary metabolites profile and its in vitro antioxidant activities were also conducted. Results revealed that serum cTnI and LDH were significantly elevated by the DOX treatment. Similarly, activities of tissue SOD, CAT, GST, and GSH levels were profoundly reduced, while GPx activity and MDA levels were profoundly increased by DOX treatment. These biochemical changes were associated with microthrombi formation in the DOX-treated cardiac tissues on histological examination. However, oral pretreatments with 100-400 mg/kg/day of IGESE dissolved in 5% DMSO in distilled water significantly attenuated increases in the serum cTnI and LDH, prevented significant alterations in the serum lipid profile and the tissue activities and levels of oxidative stress markers while improving cardiovascular disease risk indices and DOX-induced histopathological lesions. The in vitro antioxidant studies showed IGESE to have good antioxidant profile and contained 56 major secondary metabolites prominent among which are γ-sitosterol, Phytol, neophytadiene, stigmasterol, vitamin E, hexadecanoic acid and its ethyl ester, Phytol palmitate, campesterol, lupeol, and squalene. Overall, both the in vitro and in vivo findings indicate that IGESE may be a promising prophylactic cardioprotective agent against DOX-induced cardiotoxicity, at least in part mediated via IGESE’s antioxidant and free radical scavenging and antithrombotic mechanisms.
1. Introduction

Doxorubicin (otherwise known as Adriamycin) is one of the antibiotic cytotoxic agent belonging to the anthracycline class of anticancer agents [1]. Doxorubicin is known to bind to and intercalate with DNA, thereby inhibiting the resealing action of topoisomerase II during normal DNA replication needed for cancer cell division and growth [2–5]. Doxorubicin is often used in clinical setting in combination with other classes of anticancer agents as “chemo cocktail” in the management of various types of solid and blood cancers such as breast and ovarian, leukemia (acute myelogenous leukemia (AML) and acute lymphoblastic leukemia), Hodgkin lymphoma, non-Hodgkin lymphoma, Wilms’ tumor, neuroblastoma, and sarcoma [6–8]. For example, for breast cancer management, doxorubicin is typically combined and given with cyclophosphamide; for lymphomas and leukemias, it is combined with other cytotoxic agents to make regimens like CHOP (cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone), R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone), and ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) [9–12]. However, the clinical use of doxorubicin have been reported to be associated with major common side effects such as pain at the injection site, anorexia, fever, nausea and vomiting, stomatitis, dyspnea, nose bleeding, alopecia, immunosuppression, weight gain, hepatic and renal injuries, and severe cardiotoxicity [3, 13], while its occasional side effects include hyperuricemia, heart failure, pericardial effusion, cardiomyopathy, conjunctivitis, and skin rashes [14, 15]. Of these side effects, cumulative and dose-related cardiomyopathy and heart failure are of grave concerns to cancer patients and managing physicians alike, thus, limiting its clinical use [16–18]. Although the pathogenesis of doxorubicin-induced cardiotoxicity has been reported to be complex and fuzzy, the pivotal role of iron-related inflammation, cardiomyopathy, and iron. It is also a rich source of flavonoids (quercetin and kaempferol), ellagic acid, mono-, di-, and tri-O-methyl-ellagic acids, and their glycosides which are potent antioxidants [35, 36].

Phytochemical analysis of its seeds showed that it contains tannins, alkaloids, flavonoids, cardiac glycosides, steroids, carbohydrate, volatile oils, and terpenoids [33, 37, 38] and its proximate composition of moisture 1.4 ± 0.11%, ash 6.8 ± 0.12%, crude lipid 7.9 ± 0.01%, crude fiber 21.6 ± 0.45%, and crude protein 5.6 ± 0.20% [33]. Pure compounds already isolated from the seed extract of include: methyl 2-[2-formyl-5-(hydroxymethyl)-1 H-pyrroliyl]-propanoate, kaempferol-3-O-β-D-6” (p-coumaroyl) glucopyranoside and lupeol (β-lup-20(29)-en-3-ol). Erstwhile, the antioxidant property of Irvingia gabonensis seed extract has been largely attributed to its high lupeol content [39].

In view of the above, the current study was designed at evaluating the possible protective effect of the crude non-defatted ethanol seed extract of Irvingia gabonensis against doxorubicin-mediated cardiotoxicity in rats using cardiac injury markers, oxidative stress markers, and histopathology results as endpoint outcomes.

2. Materials and Methods

2.1. Extraction Process and Calculation of Percentage Yield. For Irvingia gabonensis seed extraction, 3 kg of pulverized Irvingia gabonensis dried seeds was macerated in 12 L of absolute ethanol for 72 hours after which it was continuously stirred for 1 hour before it was filtered using 180 mm of filter paper. The filtrate was then concentrated at 40°C to complete dryness using rotary evaporator. The dark-colored, oily paste-like residue left behind was weighed, stored in air- and water-proof container which was kept in a refrigerator at 4°C. This extraction process was repeated for two more times. From the stock, fresh solutions were made whenever required.

% yield was calculated as \( \frac{\text{weight of crude extract obtained (g)}}{\text{weight of pulverized dry seed extracted (g)}} \times 100 \).
2.2. Preliminary Qualitative Phytochemical Analysis of IGESE. The presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides, and reducing sugars in IGESE was detected by the simple and standard qualitative methods described by Trease and Evans [40] and Sofowora [41].

2.3. Preliminary Quantitative Determination of Secondary Metabolites in and Phytoscan of IGESE. Preliminary quantitative analysis of the secondary metabolites (including phenol, flavonoids, tannin, terpenoids, steroids, reducing sugars, saponin, and phlobatannin) in IGESE was done using methods earlier described by Olorundare et al. [42]. Similarly, using gas chromatography-mass spectrophotometer (GC-MS) for phytoscan, the relative abundance of the secondary metabolites in IGESE was done using the procedures earlier described by Olorundare et al. [42].

2.4. In Vitro Antioxidant Studies of IGESE. DPPH scavenging activity, FRAP, and nitric oxide scavenging activities of IGESE were determined using the procedures earlier described by Olorundare et al. [42].

2.5. Experimental Animals. Young adult male Wistar Albino rats (aged 8-10 weeks old and body weight: 140-160 g) used in this study were obtained from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria, after an ethical approval (UERC Approval number: UERC/ASN/2020/2022) was obtained from the University of Ilorin Ethical Review Committee for Postgraduate Research. The rats were handled in accordance with international principles guiding the Use and Handling of Experimental Animals [43]. The rats were maintained on standard rat feed (Ladokun Feeds, Ibadan, Oyo State, Nigeria) and potable water which were made available ad libitum. The rats were maintained at an ambient temperature between 28 and 30°C, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12/12 hours of alternating light and dark periodicity.

2.6. Measurement of Body Weight. The rat body weights were taken at the beginning and last of the experiment using a digital rodent weighing scale (®Virgo Electronic Compact Scale, New Delhi, India). The obtained values were expressed in grams (g).

2.7. Induction of DOX-Induced Cardiotoxicity and Treatment of Rats. Prior to commencement of the experiment, rats were randomly allotted into 7 groups of 7 rats per group such that the weight difference between and within groups was not more than ±20% of the average weight of the sample population of rats used for the study. However, the choice of the therapeutic dose range of 100, 200, and 400 mg/kg/day of IGESE was made based on the result of the orientation studies conducted.

Treatments of rats with distilled water, 100-400 mg/kg/day of IGESE in 5% DMSO distilled water, 20 mg/kg/day of vitamin C (standard antioxidant drug) for 10 days, and subsequent treatment with single intraperito-

neal dose (15 mg/kg) doxorubicin in 0.9% normal saline on day 11 are as indicated in Table 1.

2.8. Collection of Blood Samples. 72 hours postdoxorubicin injection, overnight fasted rats were humanely sacrificed under light inhaled diethyl ether anesthesia, and whole blood samples were collected directly from the heart with fine 21G injectable needle and 5 ml syringe without causing damage to the heart tissues. The rat heart, liver, kidneys, and testes were carefully identified, harvested, and weighed.

2.9. Bioassays. Blood samples collected into 10 ml plain sample bottles were allowed to clot at room temperature for 6 hours and then centrifuged at 5000 rpm to separate clear sera from the clotted blood samples. The clear samples were obtained for assays of the following biochemical parameters: serum cardiac troponin I, LDH, TG, TC, and cholesterol fractions (HDL-c, LDL-c) using estimated standard bioassay procedures and commercial kits.

2.10. AI and CRI Calculation. AI was calculated as LDL − c (mg/dl) + HDL − c (mg/dl) [44], while CRI was calculated as TC (mg/dl) + HDL − c (mg/dl) [45].

2.11. Determination of Cardiac Tissue Antioxidant Profile. After the rats were sacrificed humanely under inhaled diethyl ether, the heart was harvested en bloc. The heart was gently and carefully divided into two halves (each consisting of the atrium and ventricle) using a new surgical blade. The left half of the heart was briskly rinsed in ice-cold 1.15% KCl solution in order to preserve the oxidative enzyme activities of the heart before being placed in a clean sample bottle which itself was in an ice-pack filled cooler. This is to prevent the breakdown of the oxidative stress enzymes in these organs.

Activities of cardiac tissue oxidative stress markers such as SOD, CAT, MAD, GSH, GPx, and GST were assays using methods earlier described by Olorundare et al. [42].

2.12. Histopathological Studies. The right halves of the seven randomly selected rats from each treatment and control groups were subjected to histopathological examinations; the choice of the right ventricle was based on its reported most susceptibility to doxorubicin toxicity of the four heart chambers. The dissected right heart half was briskly rinsed in normal saline and then preserved in 10% formo-saline. It was then completely dehydrated in 100% ethanol before it was embedded in routine paraffin blocks. 4-5 μm thick sections of the cardiac tissue were prepared from these paraffin blocks and stained with hematoxylin-eosin. These were examined under a photomicroscope connected to a host computer for any associated histopathological lesions.

2.13. Statistical Analysis. Data were presented as mean ± S.E.M. of four observations for the in vitro studies and mean ± S.D. of seven observations for the in vivo studies, respectively. Statistical analysis was done using a two-way analysis of variance followed by the Student-Newman-Keuls test on GraphPad Prism Version 5. Statistical significance was considered at p < 0.05, p < 0.001, and p < 0.0001.
3. Results

3.1. % Yield. Complete extraction of Irvingia gabonensis ethanol seed extract in absolute ethanol resulted in an average yield of 4.31%, which was a very dark brown, oily, and sweet-smelling paste-like residue that was soluble in methanol and ethanol but not in water.

3.2. Preliminary Qualitative Phytochemical Analysis of IGESE. This shows the presence of phenol, flavonoids, tannin, terpenoids, steroids, and reducing sugars, while saponin and phlobatannin were absent.

3.3. Preliminary Quantification of the Secondary Metabolites in IGESE. Preliminary quantitative analysis of IGESE showing the relative abundance and quantification of secondary metabolites (expressed in mg/100 g of dry IGESE) shows the presence of phenol (57.18 ± 0.05), flavonoids (18.19 ± 0.07), alkaloids (50.51 ± 0.17), steroids (47.47 ± 0.03), tannin (41.60 ± 0.03), and reducing sugars (65.64 ± 0.23) (Table 2).

3.4. Phytoscan for Secondary Metabolites in IGESE Using Gas Chromatography-Mass Spectrometry. The presence and relative abundance of fifty-six (56) major secondary metabolites in IGESE obtained through gas chromatography-mass spectrometry and phytoscan based on CAS Library search included 4,6-di-O-methyl-alpha-d-galactose (27.08%), n-hexadecanoic acid (5.51%), undecanoic acid (5.08%), 9,12,15-octadecatrienoic acid, (Z,Z,Z) (4.84%), γ-sitosterol (4.18%), Phytol (3.84%), neophytadiene (3.77%), ethyl 9,12,15-octadecatrienoate (3.65%), stigmasterol (3.03%), vitamin E (2.91%), hexadecanoic acid, ethyl ester (2.51%), Phytol palmitate (1.92%), campesterol (1.34%), lupeol (1.22%), 9,12-octadecadienoic acid, (Z,Z) (0.96%), octadecanoic acid, ethyl ester (0.91%), lup-20(29)-en-3-one (0.84%), β-amyrone (0.82%), phenol (0.82%), 1-hexacosanol (0.77%), pyrrolidine, 1-(1-cyclohexen-1-yl)-(0.71%), triacontyl acetate (0.66%), octadecanolic acid, 2,3-dihydroxypropyl ester (0.59%), γ-tocopherol (0.35%), 1,2-bis(trimethylsilyl) benzene (0.34%), and squalene (0.26%) (Table 3 and Figure 1).

3.5. In Vitro Antioxidant Profiling of IGESE

3.5.1. Determination of DPPH Scavenging Activity of IGESE. Table 4 shows the in vitro DPPH scavenging activities of 25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml of IGESE in comparison with those of corresponding doses of the standard antioxidant drug (Vit. C) used. IGESE’s DPPH scavenging activities were significantly (p < 0.001 and p < 0.0001) dose related at 75 μg/ml and 100 μg/ml, and these were comparable to that of Vit. C (Table 4).

3.5.2. Determination of NO Scavenging Activity of IGESE. Table 5 shows the in vitro NO scavenging activities of 25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml of IGESE in comparison with those of corresponding doses of the standard antioxidant drug (Vit. C). IGESE’s NO scavenging activities of the extract were significantly (p < 0.001, p < 0.0001) dose related and comparable to that of Vit. C at 75 μg/ml and 100 μg/ml of IGESE (Table 5).

3.5.3. Determination of FRAP Activity of IGESE. Table 6 shows IGESE’s in vitro ferric reducing activity power of 25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml in comparison with those of corresponding doses of the standard antioxidant drug. Again, IGESE’s FRAP activities were significantly (p < 0.05, p < 0.001, p < 0.0001) dose dependent and comparable to that of Vit. C especially at 50 μg/ml, 75 μg/ml, and 100 μg/ml of IGESE (Table 6).
| Pl# | RT   | Area (%) | Library/IDRef#                     | CAS#               | Quality (%) |
|-----|------|----------|-----------------------------------|--------------------|-------------|
| 1.  | 4.069| 0.1378   | Ethanol, 2-(ethylamino)-           | 000110-73-6        | 80          |
| 2.  | 4.906| 0.0411   | Oxime-, methoxy-phenyl-            | 1000222-86-6       | 91          |
| 3.  | 5.137| 0.1764   | 1,2-Cyclopentanediene             | 003008-40-0        | 78          |
| 4.  | 5.455| 0.0811   | Cyclotetrasiloxane, octamethyl-    | 000556-67-2        | 83          |
| 5.  | 5.721| 0.8170   | Phenol                            | 000108-95-2        | 90          |
| 6.  | 5.905| 0.1070   | Phenol                            | 000108-95-2        | 60          |
| 7.  | 8.291| 0.1399   | Z,Z-7,11-Hexadecadien-1-ol        | 1000131-01-4       | 50          |
| 8.  | 8.458| 0.0616   | Cyclotetrasiloxane, octamethyl-    | 000556-67-2        | 64          |
| 9.  | 10.387| 0.0843 | Naphthalen-4a,8a-imine, octahydro- | 005735-21-7        | 50          |
| 10. | 10.503| 0.7119 | Pyrrolidine, 1-(1-cyclohexen-1-yl)- | 001125-99-1        | 50          |
| 11. | 11.288| 0.1380 | Cycloheptasiloxane, tetradecamethyl- | 000107-50-6        | 60          |
| 12. | 12.137| 0.4489 | 4-Methyl-2,5-dimethoxybenzaldehyde | 004925-88-6        | 60          |
| 13. | 13.125| 5.0814 | Undecanoic acid                    | 000112-37-8        | 53          |
| 14. | 14.516| 3.7713 | Neophytadiene                      | 000504-96-1        | 89          |
| 15. | 15.088| 27.0790 | 4,6-di-O-methyl-alpha-d-galactose | 024462-98-4        | 52          |
| 16. | 15.695| 5.5072 | n-Hexadecanoic acid                | 000057-10-3        | 99          |
| 17. | 15.816| 2.5123 | Hexadecanoic acid, ethyl ester     | 000628-97-7        | 98          |
| 18. | 16.116| 0.0474 | Heptadecanoic acid                 | 000506-12-7        | 55          |
| 19. | 16.595| 0.1190 | Heptadecanoic acid, ethyl ester    | 014010-23-2        | 60          |
| 20. | 16.774| 3.8358 | Phytole                           | 000150-86-7        | 91          |
| 21. | 17.063| 4.8375 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 000463-40-1        | 99          |
| 22. | 17.185| 3.6541 | Ethyl 9,12,15-octadecatrienoate    | 1000336-77-4       | 98          |
| 23. | 17.352| 0.9067 | Octadecanoic acid, ethyl ester    | 000111-61-5        | 98          |
| 24. | 17.508| 0.3478 | 14-Pentadecenoic acid             | 017351-34-7        | 86          |
| 25. | 18.420| 0.1330 | Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl- | 024903-95-5        | 55          |
| 26. | 18.605| 0.0633 | Cis-vaccenic acid                 | 000506-17-2        | 91          |
| 27. | 18.761| 0.1262 | Heptadecanoic acid, ethyl ester    | 014010-23-2        | 70          |
| 28. | 19.264| 0.0555 | Cyclopentadecanone, 2-hydroxy-    | 004727-18-8        | 90          |
| 29. | 19.425| 0.173  | Ethyl 9-hexadecenoate            | 054546-22-4        | 58          |
| 30. | 19.599| 0.594  | Octadecanoic acid, 2,3-dihydroxypropyl ester | 000123-94-4        | 87          |
| 31. | 19.818| 0.0961 | 1,4-benzenedicarboxylic acid, mono(1-methyl) ester | 1000400-56-6       | 52          |
| 32. | 19.934| 0.0379 | Cis-9-tetradecenoic acid, heptyl ester | 1000405-20-8     | 70          |
| 33. | 20.078| 0.1537 | Docosanoic acid, ethyl ester      | 005908-87-2        | 93          |
| 34. | 20.251| 0.0452 | 18-nonadecenoic acid              | 076998-87-3        | 64          |
| 35. | 20.742| 0.3606 | 1,3,12-nonadecatriene              | 1000131-11-1       | 64          |
| 36. | 20.887| 0.1046 | 2-methyl-Z,Z,3,13-octadecadienol  | 1000130-90-5       | 55          |
| 37. | 21.510| 0.2565 | Squalene                          | 000111-02-4        | 90          |
| 38. | 22.844| 0.3462 | y-Tocopherol                      | 007616-22-0        | 98          |
| 39. | 23.052| 0.6599 | Triacylacetate                     | 041755-58-2        | 95          |
| 40. | 23.341| 2.9085 | Vitamin E                         | 000059-02-9        | 99          |
| 41. | 24.040| 1.3362 | Campesterol                       | 000474-62-4        | 99          |
| 42. | 24.277| 3.0258 | Stigmasterol                      | 000083-48-7        | 99          |
| 43. | 24.427| 0.7673 | 1-hexacosanol                     | 005006-52-5        | 91          |
| 44. | 24.542| 0.1545 | Hexadecanoic acid, 2-hydroxy-,methyl ester | 016742-51-1        | 59          |
| 45. | 24.750| 4.1775 | y-Sitostanol                      | 000083-47-6        | 99          |
| 46. | 24.843| 0.8204 | B-Amyrnone                        | 000638-97-1        | 94          |
| 47. | 25.241| 0.8408 | Lup-20(29)-en-3-one               | 001617-70-5        | 97          |
| 48. | 25.443| 1.2194 | Lupeol                            | 000545-47-1        | 58          |
Table 3: Continued.

| Pk# | RT  | Area (%) | Library/ID Ref# | CAS#       | Quality (%) |
|-----|-----|----------|-----------------|------------|-------------|
| 49  | 25.559 | 0.0751   | Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl- | 1000258-63-4 | 50          |
| 50  | 25.969 | 0.3833   | Stigmaster-4-en-3-one | 001058-61-3 | 87          |
| 51  | 26.431 | 0.0624   | 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- | 054965-43-4 | 50          |
| 52  | 26.829 | 1.9166   | Phytol palmitate | 1000413-67-8 | 96          |
| 53  | 27.170 | 0.1216   | 1,4-Bis(trimethylsilyl)benzene | 013183-70-5 | 78          |
| 54  | 27.592 | 0.0250   | 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- | 054965-43-4 | 50          |
| 55  | 28.463 | 0.3430   | 1,2-Bis(trimethylsilyl)benzene | 017151-09-6 | 76          |
| 56  | 29.376 | 0.9577   | 9,12-Octadecadienoic acid (Z,Z)- | 000060-33-3 | 50          |

pk#: peak number; RT: retention time; Area%: percentage area covered; Library/ID Ref#: library/identification number; CAS#: chemical abstract scheme number.

Figure 1: GC-MS analysis showing the relative abundance of the secondary metabolites in IGESE.

Table 4: In vitro DPPH scavenging activity (% inhibition) of 25-100 μg/ml of IGESE and Vit. C.

| Drug      | Graded doses |
|-----------|--------------|
|           | 25 μg/ml    | 50 μg/ml | 75 μg/ml | 100 μg/ml |
| IGESE     | 14.59 ± 0.31 | 43.53 ± 0.19 | 67.98 ± 0.38 | 75.44 ± 0.51 |
| Vit. C    | 45.06 ± 0.28 | 56.55 ± 0.55 | 76.92 ± 0.31 | 89.83 ± 0.21 |

* and ** represent significant increases at p < 0.05, p < 0.001, and p < 0.0001, respectively, when compared to the baseline value at 25 μg/ml.

Table 5: In vitro nitric oxide (NO) scavenging activity of 25-100 μg/ml of IGESE and Vit. C.

| Drug      | Graded doses |
|-----------|--------------|
|           | 25 μg/ml    | 50 μg/ml | 75 μg/ml | 100 μg/ml |
| IGESE     | 13.55 ± 0.70 | 39.98 ± 0.70 | 68.39 ± 0.32 | 77.09 ± 0.13 |
| Vit. C    | 47.89 ± 0.14 | 63.09 ± 0.24 | 76.07 ± 0.47 | 84.91 ± 0.31 |

* and ** represent significant increases at p < 0.05, p < 0.001, and p < 0.0001, respectively, when compared to the baseline value at 25 μg/ml.

Table 6: In vitro FRAP activities of 25-100 μg/ml of IGESE and Vit. C.

| Drug      | Graded doses |
|-----------|--------------|
|           | 25 μg/ml    | 50 μg/ml | 75 μg/ml | 100 μg/ml |
| IGESE     | 0.08 ± 0.00 | 0.13 ± 0.04 | 0.28 ± 0.00 | 0.48 ± 0.00 |
| Vit. C    | 0.24 ± 0.00 | 0.38 ± 0.00 | 0.48 ± 0.00 | 0.63 ± 0.00 |

* and ** represent significant increases at p < 0.05, p < 0.001, and p < 0.0001, respectively, when compared to the baseline value at 25 μg/ml.

Table 7: MDA levels.

3.6. Effect of IGESE on the Cardiac Tissue Oxidative Stress Markers (GSH, GST, GPx, SOD, CAT, and MDA) of DOX-Treated Rats. Intraperitoneal injection of DOX to rats resulted in significant (p < 0.05, p < 0.001, and p < 0.0001) decreased activities of SOD, CAT, GPx, GST, and GSH levels while significantly increasing (p < 0.001) MDA activities (Table 7). However, oral pretreatment with IGESE significantly (p < 0.05, p < 0.001, and p < 0.0001) attenuated the alterations in the activities of these cardiac tissue enzyme markers. Similarly, IGESE pretreatment significantly (p < 0.001 and p < 0.0001) and dose dependently reduced MDA levels (Table 7).
respectively, when compared to untreated positive control (DOX-treated only, Group III).

Acute intraperitoneal LDL-c) Level of DOX-Treated Rats.

3.8. Effects of IGESE on the Serum Lipids (TG, TC, HDL-c, LDL-c) Level of DOX-Treated Rats. Acute intraperitoneal DOX injection resulted in significant (p < 0.0001) increases in the serum LDH and cTnI levels when compared to that of untreated negative control (Group I) values (Table 8). However, with oral pretreatments with 100–400 mg/kg/day of IGESE significantly attenuated (p < 0.05, p < 0.001, and p < 0.0001) increases in the serum cTnI and LDL levels dose dependently (Table 8), and these attenuations were comparable to that induced by oral pretreatment with 20 mg/kg/day of Vit. C (Table 8).

3.9. Effect of Oral IGESE Pretreatment on Cardiovascular Risk Indices (AI and CRI) of DOX-Treated Rats. Acute intraperitoneal injections with DOX resulted in significant (p < 0.001) increases in AI and CRI values when compared to Groups I and II values (Table 9). However, with oral pretreatment with 100–400 mg/kg/day of IGESE, there were significant (p < 0.05, p < 0.001, and p < 0.0001) dose-related decreases in the AI and CRI values with similar effect induced by oral pretreatments with 20 mg/kg/day of Vit. C (Table 9).

3.10. Histopathological Studies of the Effect of IGESE Oral Pretreatment on DOX-Intoxicated Treated Heart. Figure 2 is a photomicrograph of a cross-sectional representative of DOX-only treated heart showing myocyte congestion and

Table 8: Effect of 100–400 mg/kg/day of IGESE on serum LDH and cardiac troponin I (cTnI) levels in DOX-intoxicated rats.

| Treatment groups | LDH (U/L) | cTnI (ng/ml) |
|------------------|-----------|-------------|
| I                | 4347 ± 596.4 | 3.4 ± 1.1   |
| II               | 4338 ± 238.1 | 3.7 ± 1.1   |
| III              | 8151 ± 441.0c | 40.5 ± 3.5c |
| IV               | 4887 ± 217.5b | 11.4 ± 3.5c |
| V                | 4737 ± 260.2a | 25.5 ± 3.3c |
| VI               | 4188 ± 229.2b | 19.8 ± 2.4b |
| VII              | 3679 ± 346.1c | 14.8 ± 1.1c |

b and c represent significant increases at p < 0.05 and p < 0.001, respectively, when compared to untreated positive control (DOX-treated only, Group III) values. 
AST, ALT, CK-MB, LDH, and cTnI which leak from cardiac tissue damage to the bloodstream due to their tissue specificity and serum catalytic activity [46]. DOX administration may result in the damage to the myocardial cell membrane or make myocytes more permeable, resulting in the leakage of the diagnostic cardiac enzyme markers cardiac AST, ALT, CK-MB, LDH, and cTnI into the bloodstream and their high circulating levels. In the present study, DOX-mediated cardiotoxicity was fully established as evidenced by the profound elevations in the serum cTnI and LDH levels which is in complete agreement with previous studies [47–52]. With oral IGESE pretreatments, the serum levels of cTnI and LDH were profoundly attenuated toward normal serum level indicating the ameliorative potential of IGESE in DOX-mediated cardiotoxicity. These effects were probably mediated through high antioxidant and/or free radical scavenging activities of IGESE on the myocardium, thus reducing the damaging effects of DOX to the cardiac muscle fibers, subsequently minimizing the leakage of such enzymes in the serum. Similarly, ROS-mediated mechanism is one of the proposed DOX-mediated cardiotoxicity mechanisms, leading to oxidative stress that causes cardiomyopathy [53]. Oxidative stress has been reported to increase lipid peroxidation as indicated by an increase in MDA levels and altered enzymatic and nonenzymatic antioxidant systems [54, 55]. In this study, MDA level was profoundly increased by DOX treatment, while DOX treatment also suppressed the cardiac tissue activities of SOD, CAT, GPx, GST, and GSH levels in the treated rats in agreement with other studies. These altered biochemical alterations were supported by histological lesions characterized by myocyte congestion and coronary intravascular microthrombi formation. DOX has been previously reported to profoundly reduce vascular blood flow, disintegrate vascular endothelium, and promote GPIIb/IIIa-mediated platelet adhesion and aggregation, all resulting in microthrombosis [56–58]. The fact that IGESE prevented microthrombi formation in DOX-treated coronary vasculature as evidenced by histopathological results of this study highlighted the possible inherent antithrombotic potential of IGESE; although, further studies are still needed in this respect in order to validate this hypothesis. However, IGESE profoundly attenuated

| Groups | TG (mmol/l) | TC (mmol/l) | HDL-c (mmol/l) | LDL-c (mmol/l) |
|--------|-------------|-------------|----------------|---------------|
| I      | 1.2 ± 0.1   | 2.0 ± 0.1   | 0.7 ± 0.0      | 0.7 ± 0.0     |
| II     | 1.1 ± 0.1   | 1.8 ± 0.1   | 0.6 ± 0.0      | 0.6 ± 0.1     |
| III    | 0.9 ± 0.1a+ | 2.7 ± 0.3c+ | 0.7 ± 0.0      | 1.6 ± 0.2c+   |
| IV     | 1.2 ± 0.1d+ | 1.4 ± 0.2d+ | 0.8 ± 0.1d+    | 0.4 ± 0.2d−   |
| V      | 1.0 ± 0.2   | 2.4 ± 0.2d− | 0.8 ± 0.1d+    | 1.2 ± 0.1d−   |
| VI     | 1.3 ± 0.2d+ | 2.3 ± 0.2d+ | 0.9 ± 0.1b+    | 1.1 ± 0.2d−   |
| VII    | 1.5 ± 0.6e+ | 1.6 ± 0.1c− | 0.6 ± 0.0      | 0.6 ± 0.1f−   |

*p* represents a significant decrease at *p* < 0.05 when compared to (Groups I and II) values, while "++" represents a significant increase at *p* < 0.0001 when compared to Groups I and II values; "−" and "+" represent significant decreases at *p* < 0.05 and *p* < 0.001, respectively, when compared to DOX-only treated (Group III) values; "−" and "+" represent significant increases at *p* < 0.05 and *p* < 0.001, respectively, when compared to untreated positive control (DOX-only treated) (Group III) values, respectively.

![Figure 2: A cross-sectional representative of 15 mg/kg of DOX-only intoxicated rat cardiac tissue showing antemortem coronary artery microthrombi and congested cardiomyocytes suggestive of coronary intravascular thrombosis (×400 magnification, Hematoxylin and Eosin stain).](image-url)
significant alterations in the cardiac tissue oxidative markers whose activities were significantly suppressed by DOX intoxication. *IGESE* has the tendency to neutralize ROS like superoxide radicals, singlet oxygen, nitric oxide, and peroxynitrite, thereby reducing the damage to lipid membranes [39]. Similarly, oral *IGESE* pretreatments profoundly improved and reversed the DOX-induced histological lesions especially at 200 mg/kg/day and 400 mg/kg/day of *IGESE* pretreatments.

The effects of DOX on serum lipids are also significant. DOX has been reported to cause hyperlipidemia (which include increased serum cholesterol, triglyceride, LDL-c, and FFAs) [59–64] and increases cardiovascular disease risk [65]. This hyperlipidemia is thought to be mediated via downregulation of PPAR-γ and subsequently affect GLUT4 and FAT/CD36 expression resulting in glucose and fatty acid transporters expression and causing hyperglycemia and hyperlipidemia [65]. *Irvingia gabonensis* seeds have been reported to induce weight loss, antihyperlipidemia, and reduced cardiovascular disease risk factors in both animal [59–64] and human studies [66–72] which were reportedly mediated via downregulation of the PPAR-γ and leptin genes and upregulation of the adiponectin gene mechanisms [67]. Thus, the results of this study are in tandem with those of earlier studies.

The GC-MS analysis and phytoscan of *IGESE* are also notably significant. *IGESE* is shown to contain high contents of 4,6-di-O-methyl-alpha-d-galactose, n-hexadecanoic acid, undecanoic acid, 9,12,15-octadecatrienoic acid, γ-sitosterol, phytol, neophytadiene, ethyl 9,12,15-octadecatrienoate, stigmasterol, vitamin E, hexadecanoic acid ethyl ester, Phytyl palmitate, campesterol, and lupeol. Phytosterols such as sitosterol, stigmasterol, campesterol, and phytols have been reported to effectively mitigate lipid peroxidation through antioxidant and free radical scavenging mechanisms and physically stabilize cell membrane [73] as well as effectively
lowered cholesterol especially the LDL-c fraction [74–78]. Similarly, stigmasterol, γ-sitosterol, lupeol, lupeol acetate, and α-amyрин are known to exhibit other important pharmacological activities such as anticancer, anti-inflammatory, and antibacterial activities [79]. Lupeol in particular is known to mediate anti-inflammatory, antimicrobial, anti-protozoal, antiproliferative, anti-invasive, antiangiogenic, and cholesterol-lowering activities [79, 80]. Phytol is an important diterpene that possesses antimicrobial, antioxi-
dant, and anticancer properties [81, 82]. Hexadecanoic acid is known to exhibit strong antimicrobial and anti-inflammatory activity [83]. Squalene, a triterpene, is a natural antioxidant [84], possessing various other pharmacological properties including antimicrobial property [85, 86]. Neophytadiene is a good analgesic, antipyretic, anti-inflamma-
tory, antimicrobial, and antioxidant compound [87, 88]. Thus, the presence of stigmasterol, γ-sitosterol, lupeol, phyl-
tols, and neophytadiene in high amounts in IGESE could be responsible for the cholesterol-lowering, antioxidant, and antilipoperoxidation activities of IGESE in DOX-mediated cardiotoxic rats. Similarly, flavonoids, steroids, cardiac gly-
cosides, tannin, and saponin have been reported to elicit antithrombotic activities [89–91], and more specifically, plant-derived sitosterol has been reported to have antico-
agulant and thrombus-preventing activities in mice [78, 92, 93]. Thus, the presence of these phytochemicals espe-
cially steroids and tannin in high amounts in IGESE could be responsible for the observed antithrombotic action of IGESE in DOX-intoxicated rats.

5. Conclusion
Overall, results of this study showed that IGESE effectively attenuated DOX-mediated cardiotoxicity and its cardioprotective activities were mediated via antioxidant, free radical scavenging, antilipoperoxidation, and antithrombotic mechanisms.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AI:          | Atherogenic index |
| ALT:         | Alanine transaminase |
| AST:         | Aspartate transaminase |
| CAT:         | Catalase |
| CK-MB:       | Creatine kinase-MB |
| CRI:         | Coronary artery index |
| DMSO:        | Dimethyl sulfoxide |
| DNA:         | Deoxyribonucleic acid |
| DOX:         | Doxorubicin |
| DPPH:        | 1,1-diphenyl-2-picrylhydrazyl |
| FAT/CD36:    | Fatty acid translocase |
| FFAs:        | Free fatty acids |
| FRAP:        | Ferric reducing activity power |
| GC-MS:       | Gas chromatography mass spectrometer |
| GLUT4:       | Glucose transporter member 4 |
| GP1Ib/IIa:   | Glycoprotein I/IIa |
| GPx:         | Glutathione peroxidase |
| GSH:         | Reduced glutathione |
| GST:         | Glutathione S-transferase |
| HDL-c:       | High density lipoprotein cholesterol |
| i.p.:        | Intraperitoneal |
| IGES:        | Irginvia gabonensis ethanol seed extract |
| KCl:         | Potassium chloride |
| LDH:         | Lactate dehydrogenase |
| LDL-c:       | Low density lipoprotein cholesterol |
| MDA:         | Malondialdehyde |
| NO:          | Nitric oxide |
| p.o.:        | Per os |
| PPARγ:       | Peroxisome proliferator-activator receptor gamma |
| ROS:         | Reactive oxygen species |
| rpm:         | Revolution per minute |
| S.E.M.:      | Standard error of the mean |
| SOD:         | Superoxidase dismutase |
Data Availability

Answer: Yes. Comment

Conflicts of Interest

The authors have none to declare.

Authors’ Contributions

Olufunke Olorundare designed the experimental protocol for this study and was involved in the manuscript writing; Adej尤won Adeneye supervised the research, analyzed data, and wrote the manuscript; Akineye Akinsola and Olalekan Agede are postgraduate students in Olufunke Olorundare’s laboratory that performed the laboratory research under supervision; Phillip Kolo was part of the protocol design and read through the manuscript; Ikechukwu Okoye prepared the cardiac tissue slides; Sunday Soyemi and Alban Mgbehoma independently read and interpreted the cardiac tissue slides; Ralph Albrecht and Hasan Mukhtar are our collaborators in the U.S.A. who read through the manuscript.

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