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Alleviation of doxorubicin-induced nephrotoxicity by *Clerodendrum volubile* leaf extract in Wistar rats: A preliminary study

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ABSTRACT

Introduction: Doxorubicin (DOX), a well-known chemotherapeutic drug, has been reported to induce numerous toxic side effects including renal toxicity. This preliminary study was designed to investigate the ameliorative effects of methanolic leaf extract of *Clerodendrum volubile* (MECV) against DOX-induced nephrotoxicity in rats.

Methods: Thirty male rats were divided into five groups; (a) Control group: rats were given 0.9% NaCl as vehicle, (b) DOX group: a single dose of DOX (25 mg/kg; i.p.) was administered and the rats were sacrificed 4 days after DOX injection, (c-e) Methanolic extract of C. volubile (MECV)-treated DOX groups: rats were given MECV (at the doses of 125, 250 and 500 mg/kg/d), respectively for 12 consecutive days, 8 days before and 4 days after the DOX administration.

Results: DOX injection caused a significant increase ($P<0.05$) in serum creatinine and urea levels. The levels of renal antioxidant parameters: glutathione peroxidase, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione were significantly ($P<0.05$) decreased in DOX-intoxicated rats with concomitant elevation of malondialdehyde level. Pretreatment with MECV restored antioxidant status, attenuated oxidative stress and improved kidney function markers. Pre-treatment with MECV protected renal tissues against DOX-induced nephrotoxicity.

Conclusion: The ameliorative effects of *C. volubile* leaves on these renal biochemical parameters may be via its antioxidant action and may serve as a novel combination agent with DOX to limit its renal damage.

Implication for health policy/practice/research/medical education:
This study demonstrated amelioration of doxorubicin-induced nephrotoxicity by *Clerodendrum volubile* recommending its potential benefits as a novel source of combination therapeutic agent with DOX to limit its renal damage.

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Introduction

Chemotherapeutic drugs which are toxic to naturally dividing cells have found a useful role in treating tumors (1). Majority of these cytotoxic agents lack the ability to precisely differentiate between normal and cancerous cells, resulting in accumulation of these agents in healthy tissues which gives birth to deleterious clinical consequences (2). As an important antitumor, the anthracycline antibiotic, doxorubicin (DOX) is commonly used to treat a variety of malignant neoplasms including breast cancer, leukemia and solid tumors (3). However, due to its severe side effects, such as cardiotoxicity, nephrotoxicity and hepatotoxicity (4-7), the use of DOX as a chemotherapeutic agent in medicine has been limited. It is now understood that the multi-organ injury of DOX is partially due to its oxidative damage (7-9). Based on this, the use of antioxidant compounds (natural or synthetic) has been deployed as a therapeutic approach to control renal injury induced by DOX (10-12). Several studies have been conducted and documented to show the protective effects of naturally occurring substances with potent antioxidant properties against DOX- induced nephrotoxicity (13-15).

Clerodendrum volubile P. Beauv (Family: Lamiacea) is widely found growing in many deciduous forests across

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West Africa (16). Its common names are 'Obenetete' among the Urhobo and Itsekiri tribes of the Niger-Delta of Nigeria, 'Marugbo' or 'Ewe ata' among the Yoruba tribes of Ondo State in South western areas of Nigeria (17-19). In the traditional system of medicine, the plant is used for the treatment of several diseases like diabetes, rheumatism, arthritis, edema and gout (16,20-22). Some of the reported pharmacological activities of *C. volubile* leaf includes: antioxidant (21,23), hepatoprotective (24,25), antihypertensive (18), neuroprotective (26), and cancer chemopreventive (27) activities. However, to the best of our knowledge, no study has been carried out on the nephro-protective activity of the plant against DOX-induced toxicity in rats. Therefore, this preliminary study was conducted to investigate the ameliorative effects of *C. volubile* against oxidative stress toxicity induced by administration of DOX.

Materials and Methods

Drugs, chemicals and equipment

DOX (50 mg/25 mL injectable form) was purchased from Matador Dafor Pharmaceuticals Ltd, Akure (Nigeria). Other chemicals like Trichloroacetic acid, 1-chloro-2,4-dinitrobenzene, thiobarbituric acid, 5′,5′-dithiobis (2-nitrobenzoic acid), hydrogen peroxide (H_2O_2) and reduced glutathione were procured from Sigma (St Louis, MO, USA). Creatinine and urea kits were procured from Randox Laboratories Ltd (Crumlin, UK). Analytically graded chemicals were used throughout the study.

Plant collection and authentication

Fresh leaves of *C. volubile* were purchased from Oja Oba Market in Akure, Nigeria. The plant was identified and authenticated at the Department of Biology, Federal University of Technology (FUTA), Akure, Nigeria. The voucher number (FUTA/BIO/0121) was assigned.

Preparation of plant extract

Powdered leaves (500 g) of *C. volubile* was extracted with 1 L of methanol at room temperature for 24 hours and evaporated to yield the crude extract. The combined methanol extract was filtered and with the aid of rotary evaporator concentrated to obtain the crude extract from which a stock solution was prepared and administered to rats at a concentration of 200 mg/mL.

Acute toxicity study

The mean lethal dose (LD_{50}) of the methanolic extract of *C. volubile* leaf was investigated in rats (weighing 160–180 g) following the method of Chinedu et al (28).

Experimental animals and treatment

Apparently healthy adult male Wistar rats about 160-180 g in weight were purchased from the College of Medicine, Ekiti State University, Ado-Ekiti Nigeria. Animals were kept under a natural conditions (12 h light/12 h dark) throughout the experimental period. They were fed standard pellets and water, ad libitum. In this study, animal care was upheld gently in agreement with established guidelines as provided in the Guide for the Care and the Use of Laboratory Animals and in line with the University institutional Ethics Committee and Standards on animal care and experiments.

Experimental design

The rats were divided into five groups; (a) Control group: rats were given 0.9% NaCl as vehicle, (b) DOX group: A single dose of DOX (25 mg/kg; i. p.) was administered (29) and rats were sacrificed 4 days after DOX injection, (c-e) Methanolic extract of *C. volubile* (MECV)-treated DOX groups: rats were given MECV (at the dose of 125, 250 and 500 mg/kg body weight) orally, respectively viz: for 12 consecutive days; 8 days before, and 4 days after the DOX administration. At the end of treatment period, the animals were fasted overnight and then sacrificed. Blood samples were collected via cardiac puncture into dry tubes and thereafter centrifuged at 3000 × g for 10 minutes.

Tissue homogenate preparation

The kidneys were dissected, excised and rinsed in 1.15% KCl, then, blotted with filter paper and the weighed. They were then placed in an iced-cold phosphate buffer (pH 7.4) and then homogenized. The resultant kidney homogenate was subjected to centrifugation at 12 000 × g for 15 minutes at 4°C to obtain the post-mitochondrial fractions which was kept at 4°C and used for further biochemical assays.

Determination of renal functions

Blood samples were collected via cardiac puncture, centrifuged at 3000 × g for 10 minutes. Serum creatinine and blood urea nitrogen were measured as a marker of renal function, using colorimetric assay kits according to the manufacturer's instructions.

Biomarkers of oxidative damage

Assessment of lipid peroxidation

Lipid peroxidation was determined by estimating the thiobarbituric acid reactive substances formed (expressed as malondialdehyde [MDA] equivalents) following the method of Ohkawa et al (30). The level of MDA was deduced from the absorbance as described by Adám-Vizi and Seregi (31) and the unit given as nmol MDA/mg protein. The reduced GSH estimation was carried out according to Jollow et al (32).

Antioxidant enzyme activities

The activity of superoxide dismutase (SOD) was evaluated using the method of Misra and Fridovich (33). The catalase (CAT) activities were investigated according to the procedure outlined by Sinha (34). The estimation of glutathione peroxidase (GPx) activity was carried out

using the method of Lawrence and Burk (35).

Histological assessment

Kidney tissues fixed in formalin were paraffin-embedded, cut at 5 µm thickness and stained with hematoxylin and eosin (H&E) stain. Histopathological examination of the stained tissue sections was carried out by a renal histologist, who was blinded to the sample groups (36).

Data analysis

Data from this study are depicted as mean ± standard deviation. The analysis was performed by using one-way analysis of variance (ANOVA). Turkey's multiple comparison post hoc test was also carried out in all the groups using GraphPad prism 6.0 software package for Windows (37). The level of significance was placed at $P < 0.05$.

Results

Acute toxicity

The results of the acute toxicity studies revealed the non-toxic nature of *C. volubile* methanolic leaf extract. Rats administered with *C. volubile* extract appeared normal and did not show any significant changes in behavior or neurological responses up to 3000 mg/kg body weight of the extract. There was no mortality or toxicity reaction at any of the doses used until the end of the experiment (data not shown).

Effect of treatment on kidney functions

Serum urea and creatinine are indicators of kidney function. Our results showed that urea and creatinine levels were significantly increased in DOX group when compared to their corresponding control group. MECV + DOX showed decreased urea and creatinine levels in serum compared to DOX group (Figures 1 and 2).

Effect of treatment on biomarkers of oxidative damage

The antioxidant status of the kidney in normal and DOX-induced rats is shown in Table 1. The renal level of MDA increased significantly ($P < 0.05$) in the DOX-induced rats with concomitant decrease in GSH level. The pre-treatment with MECV + DOX significantly reversed the effects of DOX.

Effect of treatment on antioxidant enzymes

Administration of DOX led to significant reduction of SOD, CAT, and GPx contents compared to control group respectively in the kidney of rats. However, the concomitant administration of MECV + DOX restored enzyme activities near to the baseline valve recorded for the control group (Table 2).

Histological examination

Histopathological examination with H&E staining revealed normal renal glomeruli and cortical tubules

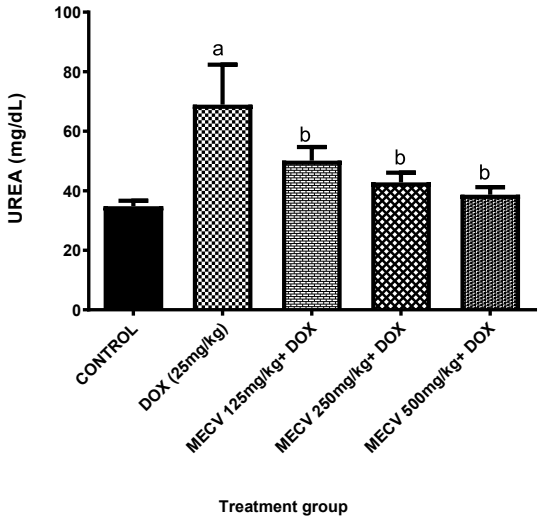

Figure 1. Effect of methanolic extract of *C. volubile* (MECV) leaves on urea levels in doxorubicin (DOX)-induced nephrotoxicity in rats. Values are expressed as mean ± SD (n = 5). [a] $P < 0.05$ compared to the control group; [b] $P < 0.05$ compared to the DOX group.

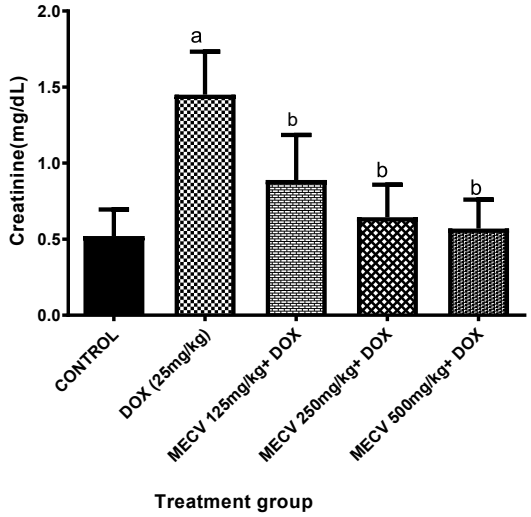

Figure 1. Effect of methanolic extract of *C. volubile* (MECV) leaves on creatinine levels in doxorubicin (DOX)-induced nephrotoxicity in rats. Values are expressed as mean ± SD (n = 5). [a] $P < 0.05$ compared to the control group; [b] $P < 0.05$ compared to the DOX group.

structures in the control group. However, DOX-treated group showed glomeruli distortion, filtration space obliterated disappear, tubules focal atrophy necrosis and exfoliation, and vascular congestion. MECV+DOX combination group showed little or no visible lesions in the observed group (Figure 3).

Discussion

Doxorubicin is an antibiotic and a strong anticancer drug with wide spectrum of therapeutic actions. However, a prominent limiting factor to the use of DOX in anti-cancer therapy is its adverse effect of nephrotoxicity.

Table 1. Effect of MECV on DOX-induced changes in GSH and MDA

Treatment group	GSH (µg/g tissue)	MDA (nmol/mg protein)
Control	4.54 ± 1.5	11.88±0.14
DOX only (25mg/kg)	1.30 ± 0.4* (71.36)[a]	16.88 ± 1.7*(29.62)[a]
MECV (125 mg/kg) + DOX (25 mg/kg)	2.16 ± 0.2** (39.81)[b]	15.18 ± 0.9 (10.07)[b]
MECV (250 mg/kg) + DOX (25 mg/kg)	2.40 ± 0.5** (45.83)[b]	13.34 ± 0.1** (20.97)[b]
MECV (500 mg/kg) + DOX (25 mg/kg)	2.78 ± 1.5** (53.24)[b]	12.55 ± 0.4** (25.65)[b]

GSH, reduced glutathione; MDA, malondialdehyde; DOX, doxorubicin; MECV, methanolic extract of *Clerodendrum volubile*.
Values are expressed as mean ± standard deviation of mean (SD) for five rats in each group.
* $P < 0.05$ compared to control group; ** $P < 0.05$ compared DOX-treated rats. Values in parenthesis represent % change; [a] % change relative to control; [b] % change relative to DOX.

Table 2. Effect of MECV on DOX-induced alterations in antioxidant parameters

Treatment group	SOD (U/mg protein)	CAT (H_2O_2 µmole consumed/min)	GPx (mol/mgprotein/min)
Control	12.69 ± 2.5	15.88 ± 2.6	136.93 ± 8.6
DOX only (25 mg/kg)	8.33 ± 1.5* (65.64)[a]	8.72 ± 1.2* (45.08)[a]	88.88 ± 4.6* (35.09)[a]
MECV (125 mg/kg) + DOX (25mg/kg)	9.95 ± 1.3** (16.23)[b]	10.73 ± 2.1** (18.73)[b]	110.36 ± 7.6** (19.46)[b]
MECV (250 mg/kg) + DOX (25 mg/kg)	10.73 ± 0.5** (22.37)[b]	12.71 ± 1.5** (31.39)[b]	116.67 ± 6.5** (23.82)[b]
MECV (500 mg/kg) + DOX (25 mg/kg)	11.24 ± 1.0** (25.89)[b]	13.86 ± 3.3** (37.09)[b]	122.12 ± 7.4** (27.22)[b]

SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde; DOX, doxorubicin; MECV, methanolic extract of *Clerodendrum volubile*.
Values are expressed as mean ± standard deviation of mean (SD) for five rats in each group.
* $P < 0.05$ compared to control group; ** $P < 0.05$ compared DOX-treated rats. Values in parenthesis represent % change; [a] % change relative to control; [b] % change relative to DOX.

Although the exact mechanism of action behind DOX-induced nephrotoxicity remains not fully elucidated, the formation of free radical, oxidative damage, and lipid peroxidation of the membranes is believed to be a major factor contributing to DOX nephrotoxicity (6,7). *C. volubile* leaf extracts have been documented to perform an important role in attenuating oxidative stress, scavenging free radicals and boosting of antioxidant defense systems (12,20,21,24,38,39). DOX-induced nephrotoxicity was observed in our study following the significant increase in serum urea and creatinine levels (Figures 1 and 2) which was confirmed by toxic histopathological changes when compared with the corresponding control group. In the diagnosis of renal injury, urea and creatinine are the commonly used diagnostic markers of nephrotoxicity (40,41). The damaging effect to the renal tissues by DOX is characterized by an increase in the level of urea and creatinine in the serum. The results from this study (Figures 1 and 2) are similar to previous studies (29,43).

Literature reports on medicinal plants and their derived bioactive components have pointed to significant improvements on DOX-induced nephrotoxicity through

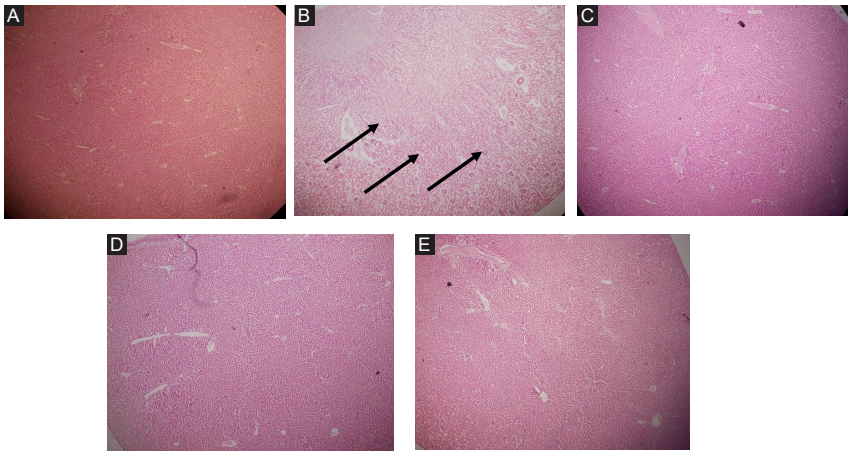

Figure 3. Histopathological changes in the rat's kidney caused by doxorubicin (DOX) and protective effect of methanolic extract of *C. volubile* (MECV) in different groups. Kidney section (×400) of the rat treated with: (A) normal saline without visible lesions; (B) DOX (25 mg/kg; i. p) showing a severe necrosis and distortion of cyto-architecture of renal tissue; (C) DOX + MECV (125 mg/kg body) showing no visible lesions; (D) DOX + MECV (250 mg/kg body), showing progressive improvement in the structure of the kidney; (E) DOX + MECV (500 mg/kg body) with no histological alterations or visible lesions.

their antioxidant and free radical scavenging activities (29,42,44). In the current study, the role of another medicinal plant (*C. volubile*) with reported antioxidant and free radical scavenging activities on DOX-induced nephrotoxicity was investigated. From our results, MECV could significantly reduce serum urea and creatinine levels compared to DOX-treated group (Figures 1 and 2). This might be due to the free radical scavenging abilities and antioxidant effects of MECV which suppressed DOX-mediated oxidative stress and tissue damage. Results from the histopathology (Figure 3) showed that DOX-treated group presented with marked damage of renal tubules showing visible lesions. This is in agreement with Kumral et al (29) and Mohebbati et al (44), who showed similar histopathological findings.

DOX has been reported to induce oxidative stress in the kidney with the evidence of an increase in lipid peroxidation and alteration in the antioxidant status indices (6). Pre-treatment with MECV obviously reduced the significant elevation in MDA content caused by DOX and is in agreement with Eleiwa et al (45) and Omobowale et al (46) who demonstrated that extracts from the plant *Spirulina platensis* decreased MDA level in the kidney of rats induced by DOX. In addition, previous report on *C. volubile* plant (21,20,23,38,39,47) have shown its potential to boost antioxidant activities and attenuate oxidative stress.

Consequently, it is not surprising that MECV is able to reduce MDA levels possibly by the presence of bioactive compounds which has been well reported to have antioxidant properties that can scavenge free radicals generation *in vivo*. These phenolic compounds are found ubiquitously in plants and they have been documented to confer many health benefits which the plants exhibit such as antioxidant, antidiabetic, anticarcinogenic, and anti-carcinogenic properties (22,48-50). Some reported bioactive compounds in *C. volubile* leaves are phenolic acids (rosmarinic acid, garlic acid, chlorogenic acid, caffeic acid) and flavonoids (quercetin, rutin, isoquercitrin) (18,19,23). Chlorogenic acid, rosmarinic and rutin are the predominant compounds present in *C. volubile* leaf. Previous studies on chlorogenic acid and rosmarinic acid have demonstrated the renoprotective abilities in xenobiotic-induced kidney damage in mice (51). The protective ability of this plant against DOX- induced kidney damage observed in this study might be as results of the presence of these bioactive components present in the leaves of *C. volubile*.

Previous studies have also shown that treatment with DOX significantly reduced renal GSH and this could bring about a short fall in the redox status pool of the cell (52). The interaction of the protein thiols and sulfhydryl groups of GSH with the resultant metabolites from DOX has been associated to this occurrence (53). The significant elevation of lipid peroxidation as shown in this study might also be responsible for the reduced GSH level. However, administration of MECV prior to DOX treatment significantly raised this reduced GSH level at the doses used in this study.

Furthermore, the activities of the renal SOD, catalase and GPx were significantly reduced in the DOX-intoxicated rats, respectively (Table 2). The decrease in the SOD activity could be due to the increased lipid peroxidation (MDA content) observed earlier (as shown in table 1), or the inactivation of the antioxidant enzymes which would ultimately result in accumulation of superoxide radicals, further initiating the lipid peroxidation process (54). The results obtained from this study corroborates report by Erukainure et al (55) who showed the antioxidant activities of an isolated iridoid glycoside from the leaves of *C. volubile* in brain and hepatic tissue. Similar antioxidant properties of *C. volubile* leaves have also been demonstrated *in vivo* by Molehin et al (24) and *in vitro* by Adefegha and Oboh (18) and Ogunwa et al (21). From histology point of view, pretreatment with MECV offered remarkable protection against kidney damage as shown in the intact renal cyto-architecture with no visible lesions.

Conclusion

In conclusion, data from this preliminary study may support the hypothesis that oxidative stress plays an important role in the mechanism of DOX-induced nephrotoxicity and that MECV has therapeutic potential in ameliorating renal injury induced by DOX possibly via antioxidant mechanism.

Author contributions

The study design, experimental and data analyses, and preparation of the manuscript was done by ORM. The author read and approved the final manuscript.

Conflict of interest

The author declares that he has no conflict of interest.

Ethical considerations

All the animals received care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by EU Directive 2010/63/EU for animal experiments. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments. The protocol for this study was approved by The Research Ethics Committee, Ebelola Bioenergetic Solutions, Osogbo Osun State Nigeria (EBS/LSR/A/7/2019/001).

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References

1. Cui J, Li C, Guo W, Li Y, Wang C, Zhang L, et al. Direct

comparison of two pegylated liposomal doxorubicin formulations: is AUC predictive for toxicity and efficacy? J Control Release. 2007;118(2):204-15. doi: 10.1016/j.jconrel.2006.12.002.
2. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol Rev. 1999;51(4):691-743.
3. Mohamed RH, Karam RA, Amer MG. Epicatechin attenuates doxorubicin-induced brain toxicity: critical role of TNF-alpha, iNOS and NF-kappaB. Brain Res Bull. 2011;86(1-2):22-8. doi: 10.1016/j.brainresbull.2011.07.001.
4. Injac R, Boskovic M, Perse M, Koprivec-Furlan E, Cerar A, Djordjevic A, et al. Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol C60(OH)24 via suppression of oxidative stress. Pharmacol Rep. 2008;60(5):742-9.
5. Lee VW, Harris DC. Adriamycin nephropathy: a model of focal segmental glomerulosclerosis. Nephrology (Carlton). 2011;16(1):30-8. doi: 10.1111/j.1440-1797.2010.01383.x.
6. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013;65(2):157-70. doi: 10.1111/j.2042-7158.2012.01567.x.
7. Yang CC, Chen YT, Chen CH, Chiang JY, Zhen YY, Yip HK. Assessment of doxorubicin-induced mouse testicular damage by the novel second-harmonic generation microscopy. Am J Transl Res. 2017;9(12):5275-88.
8. Mishra MD, Bhiwgade DA. Doxorubicin mediated oxidative stress induced degeneration of testicular tissues, causes male sterility in rats. J Cell Tissue Res. 2007;7(1):861-6.
9. Tanigaki R, Sueoka K, Tajima H, Nakabayashi A, Sato K, Asada H, et al. C-kit expression in spermatogonia damaged by doxorubicin exposure in mice. J Obstet Gynaecol Res. 2013;39(3):692-700. doi: 10.1111/j.1447-0756.2012.02006.x.
10. Hozayen WG, Abou Seif HS, Amin S. Protective effects of ruitn and / or hesperidin against doxorubicin-induced hepatotoxicity. Int J Clin Nutr. 2014;2(1):11-7.
11. Su Z, Ye J, Qin Z, Ding X. Protective effects of madecassoside against Doxorubicin induced nephrotoxicity in vivo and in vitro. Sci Rep. 2015;5:18314. doi: 10.1038/srep18314.
12. Molehin OR, Oloyede OI, Adefegha SA. Streptozotocin-induced diabetes in rats: effects of White Butterfly (*Clerodendrum volubile*) leaves on blood glucose levels, lipid profile and antioxidant status. Toxicol Mech Methods. 2018;28(8):573-86. doi: 10.1080/15376516.2018.1479476.
13. Ajith TA, Aswathy MS, Hema U. Protective effect of *Zingiber officinale* roscoe against anticancer drug doxorubicin-induced acute nephrotoxicity. Food Chem Toxicol. 2008;46(9):3178-81. doi: 10.1016/j.fct.2008.07.004.
14. Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. Food Chem Toxicol. 2010;48(1):436-40. doi: 10.1016/j.fct.2009.10.042.
15. Shalizar Jalali A, Hasanzadeh S. *Crataegus monogyna* fruit aqueous extract as a protective agent against doxorubicin-induced reproductive toxicity in male rats. Avicenna J Phytomed. 2013;3(2):159-70.
16. Burkill HM. The useful plants of west tropical Africa. 2nd ed. UK: Royal Botanical Gardens Kew; 1985; p.5.
17. Erukainure OL, Oke OV, Ajiboye AJ, Okafor OY. Nutritional qualities and phytochemical constituents of *Clerodendrum volubile*, a tropical non-conventional vegetable. Int Food Res J. 2011;18(4):1393-9.
18. Adefegha SA, Oboh G. Antioxidant and inhibitory properties of *Clerodendrum volubile* leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension. J Taibah Univ Sci. 2016;10(4):521-33. doi: 10.1016/j.jtusci.2015.10.008.
19. Molehin OR, Oloyede OI. In vitro antioxidant and sub-acute toxicity studies of aqueous extract of white butterfly (*Clerodendrum volubile*) leaves. Int J Plant Res. 2018;31(4):92-101. doi: 10.5958/2229-4473.2018.00099.X.
20. Erukainure OL, Mesaik AM, Muhammad A, Chukwuma CI, Manhas N, Singh P, et al. Flowers of *Clerodendrum volubile* exacerbate immunomodulation by suppressing phagocytic oxidative burst and modulation of COX-2 activity. Biomed Pharmacother. 2016;83:1478-84. doi: 10.1016/j.biopha.2016.09.002.
21. Ogunwa TH, Adeyelu TT, Fasimoye RY, Oyewale MB, Ademoye TA, Ilesanmi OC, et al. Phytochemical evaluation and in vitro antioxidant status of *Clerodendrum volubile* (an Indigenous medicinal plant). Pak J Pharm Res. 2016;2(2):77-88. doi: 10.22200/pjpr.2016277-88.
22. Molehin OR, Oloyede OI, Ajayi EI. GC–MS analysis of bioactive compounds in three extracts of *Clerodendrum volubile* P. Beauv leaves. J Med Plants Stud. 2017;5(5):191-5.
23. Molehin OR, Oloyede OI, Boligon AA. Comparative study on the phenolic content, antioxidant properties and HPLC fingerprinting of the leaf extracts of *Clerodendrum volubile* P. Beauv. J Appl Pharm Sci. 2017;7(3):135-40. doi: 10.7324/JAPS.2017.70322.
24. Molehin OR, Oloyede OI, Idowu KA, Adeyanju AA, Olowoyeye AO, Tubi OI, et al. White butterfly (*Clerodendrum volubile*) leaf extract protects against carbon tetrachloride-induced hepatotoxicity in rats. Biomed Pharmacother. 2017;96:924-9. doi: 10.1016/j.biopha.2017.12.005.
25. Molehin OR, Oloyede OI. Attenuation of oxidative stress and hepatic damage by white butterfly (*Clerodendrum volubile*) leaves in streptozotocin-induced diabetes in rats. J Basic Clin Physiol Pharmacol. 2018;30(1):81-9. doi: 10.1515/jbcpp-2018-0083.
26. Oboh G, Ogunruku OO, Oyeleye SI, Olasehinde TA, Ademosun AO, Boligon AA. Phenolic extracts from *Clerodendrum volubile* leaves inhibit cholinergic and monoaminergic enzymes relevant to the management of some neurodegenerative diseases. J Diet Suppl. 2017;14(3):358-71. doi: 10.1080/19390211.2016.1237401.
27. Erukainure OL, Zaruwa MZ, Choudhary MI, Naqvi SA, Ashraf N, Hafizur RM, et al. Dietary fatty acids from leaves of *Clerodendrum volubile* induce cell cycle arrest, downregulate matrix metalloproteinase-9 expression, and modulate redox status in human breast cancer. Nutr Cancer. 2016;68(4):634-45. doi: 10.1080/01635581.2016.1156714.
28. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int. 2013;20(3):224-6. doi: 10.4103/0971-6580.121674.
29. Kumral A, Giriş M, Soluk-Tekkeşin M, Olgaç V, Doğru-Abbasoğlu S, Türkoğlu Ü, et al. Effect of olive leaf extract

treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats. Pathophysiology. 2015;22(2):117-23. doi: 10.1016/j.pathophys.2015.04.002.
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3.
31. Adám-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem Pharmacol. 1982;31(13):2231-6. doi: 10.1016/0006-2952(82)90106-x.
32. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151-69. doi: 10.1159/000136485.
33. Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. J Biol Chem. 1972;247(21):6960-2.
34. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-94. doi: 10.1016/0003-2697(72)90132-7.
35. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 1976;71(4):952-8. doi: 10.1016/0006-291x(76)90747-6.
36. Disbrey BD, Rack JH. Histological Laboratory Methods.. Edinburgh: Livingstone; 1970.
37. Kirkwood BR, Sterne JAC. Essential Medical Statistics. 2nd ed. USA: Blackwell Science; 2003. p. 15-409.
38. Erukainure OL, Hafizur RM, Kabir N, Choudhary MI, Atolani O, Banerjee P, et al. Suppressive Effects of *Clerodendrum volubile* P Beauv. [Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications. Front Pharmacol. 2018;9:8. doi: 10.3389/fphar.2018.00008.
39. Olarenwaju O, Apata JT, Akinpelu BO, Akomolafe RO, Oyemitan IA, Asaolu FT, et al. Anti-inflammatory potentials, membrane stabilizing and xanthine oxidase inhibitory activities of *Clerodendrum volibule* ethanolic leaf extract on carragenaan- induced inflammation in rats. Int J Pharmacol Toxicol. 2018;6(1):7-11. doi: 10.14419/ijpt.v6i1.8410.
40. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol. 2008;48:463-93. doi: 10.1146/annurev.pharmtox.48.113006.094615.
41. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010;2(4):170-3.
42. Kim DR, Lee SY, Kim JS, Kim YG, Moon JY, Lee SH, et al. Ameliorating effect of Gemigliptin on renal injury in murine adriamycin-induced nephropathy. Biomed Res Int. 2017;2017:7275109. doi: 10.1155/2017/7275109.
43. Entezari Heravi N, Hosseinian S, Naji Ebrahimi Yazd Z, Shafei MN, Ebrahimzadeh Bideskan A, Shahraki S, et al. Doxorubicin-induced renal inflammation in rats: Protective role of Plantago major. Avicenna J Phytomed. 2018;8(2):179-87.
44. Mohebbati R, Shafei MN, Beheshti F, Soukhtanloo M, Roshan NM, Anaeigoudari A, et al. Mixed hydroalcoholic extracts of *Nigella sativa* and *Curcuma longa* improves adriamycin-induced renal injury in rat. Saudi J Kidney Dis Transpl. 2017;28(6):1270-81. doi: 10.4103/1319-2442.220880.
45. Eleiwa NZH, Galal AAA, Abd El-Aziz RM, Hussin EM. Antioxidant activity of *Spirulina platensis* alleviates doxorubicin-induced oxidative stress and reprotoxicity in male rats. Orient Pharm Exp Med. 2018;18(2):87-95. doi: 10.1007/s13596-018-0314-1.
46. Omobowale TO, Oyagbemi AA, Ajufo UE, Adejumobi OA, Ola-Davies OE, Adedapo AA, et al. Ameliorative effect of gallic acid in doxorubicin-induced hepatotoxicity in Wistar rats through antioxidant defense system. J Diet Suppl. 2018;15(2):183-96. doi: 10.1080/19390211.2017.1335822.
47. Molehin OR, Adeyanju AA, Adefegha SA, Akomolafe SF. Protocatechuic acid mitigates adriamycin-induced reproductive toxicities and hepatocellular damage in rats. Comp Clin Path. 2018;27(6):1681-9. doi: 10.1007/s00580-018-2794-2.
48. Molehin OR, Adefegha SA, Oboh G, Saliu JA, Athayde ML, Boligon AA. Comparative study on the phenolic content, antioxidant properties and HPLC fingerprinting of three varieties of *Celosia* species. J Food Biochem. 2014;38(6):575-83. doi: 10.1111/jfbc.12090.
49. Adefegha SA, Oboh G, Molehin OR, Saliu JA, Athayde ML, Boligon AA. Chromatographic fingerprint analysis, acetylcholinesterase inhibitory properties and antioxidant activities of redflower ragleaf (*Crassocephalum crepidioides*) extract. Journal of Food Biochemistry. 2016;40(1):109-19. doi: 10.1111/jfbc.12200.
50. Erukainure OL, Hafizur RM, Choudhary MI, Adhikari A, Mesaik AM, Atolani O, et al. Anti-diabetic effect of the ethyl acetate fraction of *Clerodendrum volubile*: protocatechuic acid suppresses phagocytic oxidative burst and modulates inflammatory cytokines. Biomed Pharmacother. 2017;86:307-15. doi: 10.1016/j.biopha.2016.12.035.
51. Domitrović R, Cvijanović O, Šušnić V, Katalinić N. Renoprotective mechanisms of chlorogenic acid in cisplatin-induced kidney injury. Toxicology. 2014;324:98-107. doi: 10.1016/j.tox.2014.07.004.
52. Domitrović R, Potočnjak I, Crnčević-Orlić Z, Škoda M. Nephroprotective activities of rosmarinic acid against cisplatin-induced kidney injury in mice. Food Chem Toxicol. 2014;66:321-8. doi: 10.1016/j.fct.2014.02.002.
53. Mohebbati R, Shafei MN, Soukhtanloo M, Mohammadian Roshan N, Khajavi Rad A, Anaeigoudari A, et al. Adriamycin-induced oxidative stress is prevented by mixed hydro-alcoholic extract of *Nigella sativa* and *Curcuma longa* in rat kidney. Avicenna J Phytomed. 2016;6(1):86-94.
54. Cummings J, Anderson L, Willmott N, Smyth JF. The molecular pharmacology of doxorubicin in vivo. Eur J Cancer. 1991;27(5):532-5. doi: 10.1016/0277-5379(91)90209-v.
55. Erukainure OL, Ebuehi OA, Choudhary IM, Adhikari A, Hafizur RM, Perveen S, et al. Iridoid glycoside from the leaves of *Clerodendrum volubile* beauv. shows potent antioxidant activity against oxidative stress in rat brain and hepatic tissues. J Diet Suppl. 2014;11(1):19-29. doi: 10.3109/19390211.2013.859213.