RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v23i1.3

Adeoye *et al.*/*Sokoto Journal of Veterinary Sciences*, **23**(1): 28 - 37.

Ameliorative effects of *Clerodendrum volubile* on acute kidney injury and associated cardiovascular dysfunctions in rats

BO Adeoye[1], TO Ajibade[2]*, A Adesanya[1], IO Ogunmiluyi[1], AA Oyagbemi[2], TO Omobowale[3], MO Adetona[4], OO Oguntibeju[5], MA Yakubu[6] & AA Adedapo[1]

1. *Department of Veterinary Pharmacology and Toxicology University of Ibadan, Nigeria*
2. *Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria*
3. *Department of Veterinary Medicine, University of Ibadan, Nigeria*
4. *Department of Anatomy, College of Medicine, University of Ibadan, Nigeria*
5. *Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa*
6. *Department of Environmental and Interdisciplinary Sciences, College of Science and Technology, Vascular Biology Unit, Center for Cardiovascular Diseases, College of Pharmacy, Texas Southern University, Houston, TX, USA*

*Correspondence: Tel.: +2349071842142; E-mail: toajibade@gmail.com

Publication History:
Received: 03-06-2024
Revised: 21-11-2024
Accepted: 26-11-2024

Abstract

The nephroprotective and blood pressure-lowering effects of *Clerodendrum volubile*, a plant commonly consumed in Nigeria, were evaluated in 30 rats. Glycerol was used to induce acute kidney injury, whereas the methanol leaf extract of C. volubile was used to ameliorate cardio-renal dysfunctions at the pre- and post-treatment experimental phases. Twenty-four hours post-experimentation, blood pressure parameters were measured for all groups and blood was collected for serum biochemistry. Thereafter, the rats were sacrificed, kidneys and hearts were harvested, and homogenates obtained for analysis of parameters such as protein concentrations, myeloperoxidase, as well as thiobarbituric acid reactive substances. In addition, some organs were harvested into formalin for immunohistochemistry. Results showed decreased blood pressure parameters (SBP and DBP), decreased renal functional markers (BUN and creatinine), decreased oxidative stress markers (malondialdehyde and hydrogen peroxide), but increased activities of the analysed enzymatic antioxidants and vitamin C. *volubile* extract-treated animals, compared with untreated glycerol-exposed rats. Furthermore, *C. volubile* decreased podocin and angiotensin-converting enzymes immunohistochemical expressions compared to glycerol-exposed rats. Conclusively, C. volubile at pre- and post-treatment phases caused nephroprotective and blood pressure-lowering effects in rats through anti-inflammatory, antioxidant, and inhibition of angiotensin-converting enzyme pathways.

Keywords: *Clerodendrum volubile*, Kidney, Normotension, Podocin, Rat

Introduction

Renal dysfunction is a common clinical occurrence that may be associated with significant multi-organ disturbances and deaths in humans and animals (Masina *et al.*, 2022). Physiological derangements in

the renal functional capabilities may be associated with direct and indirect altered cardiovascular system functioning due to homeostatic imbalance associated with impaired renal filtration, secretion and reabsorption processes. Acute kidney injury (AKI), defined as a sudden dysfunction characterized by sustained reduction in renal function, is a common and devastating clinical entity with increasing incidence and high mortality (Bonventre, 2007). The initiation and maintenance of AKI often involve decreased glomerular permeability associated with ischemic or nephrotoxic insults (Makris & Spanou, 2016). Despite best efforts, the management of AKI continues to pose clinical challenges that are often associated with undesirable clinical outcomes (Kellum *et al*., 2021). Several pathogenic mechanisms contribute to the development of AKI, but severe depletion of endogenous antioxidants arising due to aggravation of oxidative processes play a cardinal role (Piko *et al*., 2023).

Consequent to the prominent involvement of redox imbalance in the pathogenic mechanisms of AKI, various endogenous and synthetic antioxidants that mitigate the deleterious effects of oxidant molecules have been extensively researched for their potential beneficial roles as nephroprotective agents (Dennis & Witting, 2017). Furthermore, plants containing high antioxidant phytochemicals including phenolic compounds and carotenoids have been reported to exert protective effects on the kidney (ZarKalai *et al*., 2022). Antioxidants have the potential to intervene early in the pathogenesis of kidney injury by directly eliminating reactive oxygen species and other prooxidants *in vivo* (Dennis & Witting 2017; Omóbòwálé *et al*., 2018). Moreover, several *in vitro* and *in vivo* studies have reported potent modulatory effects of medicinal plants for a diverse array of AKI models (Panizo *et al*., 2015; Ratliff *et al*. 2016).

Clerodendrum volubile is an underutilized leafy vegetable consumed in some parts of Nigeria that has gained prominence among the medicinal plants with remarkable therapeutic uses, due to its multipurpose values in folk medicine and various nutritional attributes (Okaiyeto *et al*., 2021). Medicinally, the plant is locally used for the treatment of oedema, dropsy and arthritis, thus suggesting potent anti-inflammatory effects, with the flavonoid-rich fraction of *C. volubile* reported to attenuate arsenic-invoked hepato-renal toxicity via augmentation of the antioxidant system in rats (Ugbaja *et al*., 2021). This study was designed to evaluate the renal and cardioprotective effects of *C. volubile* in rats acutely exposed to high intramuscular injection of glycerol in the pre-and post-treatment phases.

Materials and Methods

Extraction of Clerodendrum volubile

The *C. volubile* leaves harvested in Oke-Igbo (Ondo State) were dried and blended for processing. The total weight of 116.2 g was transferred into a clean rotary flask, and 1 litre of methanol (96%) was added and allowed to soak for 24 hours. After 24 hours, the soaked sample was filtered into another clean rotary flask, evaporated kept at a constant temperature of 40 ºc C gradually increasing the pressure to distil and concentrate the extract. After concentrating the extract, the amount of crude extract was calculated.

Experimental animals

Thirty (30) laboratory male albino rats weighing between 150 and 200 g were used for this study. They were fed standard rat cubes and fresh clean water was provided *ad libitum*. The experimental rats were handled humanely, and the experimental protocol of the University of Ibadan Animal Care and Research Ethics Committee was used. The ethical approval for the study was obtained with the approval number UI-ACUREC/17/0064.

Animal groupings

The rats were randomly distributed into six groups. There were five rats in each group.

Group A: Normal Saline administered by oral gavage.

Group B: 10ml/kg 50% glycerol administered intramuscularly on the eighth day of the experiment.

Group C: Pre-treatment group administered 100 mg/kg *C. volubile* extract (dissolved in normal saline) orally for seven consecutive days and 10ml/kg 50% glycerol was administered intramuscularly on the eighth day.

Group D: Pre-treatment group administered 200 mg/kg *C. volubile* extract (dissolved in normal saline) orally for seven consecutive days and 10ml/kg 50% glycerol administered intramuscularly on the eighth day.

Group E: Post-treatment group extract. This group received 10ml/kg 50% glycerol administered intramuscularly on the first day of the experiment and was administered 100 mg/kg *C. volubile* extract (dissolved in normal saline) for seven consecutive days.

Group F: Post-treatment group extract. This group received 10ml/kg 50% glycerol administered intramuscularly on the first day of the experiment and was administered 200 mg/kg *C. volubile* extract

(dissolved in normal saline) for seven consecutive days.

Monitoring of primary haemodynamic parameters and electrocardiography

Indirect blood pressure was obtained in awake but quiescent animals initially trained and acclimatized before several repeated recordings. The most consistent readings were recorded and used for the analysis (Awoyomi *et al*., 2021). Electrocardiography was carried out in thiopental (30 mg/kg) anaesthetised rats with a twelve leads electrocardiograph, but the emphasis was placed on the leads I, II, and III as well as the augmented limb leads (Omobowale *et al*., 2018).

Biochemical evaluation

Blood samples were collected from retro-orbital venous plexus with the aid of clean heparinized capillary tubes into dry clean plain sample bottles (not containing heparin) and allowed to coagulate. The blood samples were centrifuged at 4,000 revolutions per minute (rpm) for 15 minutes. Also, the kidney and heart tissues were carefully dissected and washed in ice-cold normal saline solution, after which they were blotted with filter paper and weighed, after humane sacrifice by cervical dislocation of anaesthetised rats. They were then chopped into bits and homogenized using the homogenizing buffer (0.1M Phosphate buffer, pH 7.4) in a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 rpm for 10 minutes with a cold centrifuge at 4°C to obtain post-mitochondrial fraction (PMF). The supernatant was collected and used for biochemical analyses (Oyagbemi *et al*., 2020). The biochemical parameters evaluated in this study which included protein concentrations, nitric oxide (NO), myeloperoxidase activity, superoxide dismutase, reduced glutathione (GSH), glutathione-s-transferase, protein thiol, non-protein thiol (NPSH), protein carbonyl and thiobarbituric acid reactive substances were all determined using standard methods.

Protein concentrations were determined as described by Gornal *et al*. (1949). Briefly, 1 ml of diluted serum was added to 3 ml of the biuret reagent. The reaction mixture was incubated at room temperature for 30 minutes. The mixture was thereafter read with a spectrophotometer at 540 nm using distilled water as blank. The final value for total protein was extrapolated from the total protein standard curve.

Nitric oxide (NO) was measured as described by Olaleye *et al*. (2007) by indirectly measuring the nitrite concentration. After incubation of 100 mL of sample mixed with 100 mL of Griess reagent at room temperature for 20 minutes, the absorbance at 540 nm was measured by spectrophotometer. The concentration of nitrite in the sample was determined from a sodium nitrite ($NaNO_2$) standard curve and was expressed as µmol nitrite/mg protein.

The serum myeloperoxidase (MPO) activity was determined according to the method of Xia & Zweier (1997). Briefly, 0.1 ml of the sample was mixed with 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.53 mM *O*-dianisidine and 0.15 mM H_2O_2. The change in absorbance at 460 nm was measured every 15 s for 5 minutes with a spectrophotometer.

Superoxide dismutase (SOD) was determined by the method of Misra & Fridovich (1972). Briefly, 100 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. 0.01 ml of renal PMF was added to 2.5 ml 0.05 M carbonate buffer (pH 10.2) followed by the addition of 0.3 ml of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome for 1 minute.

Reduced glutathione (GSH) was estimated by the method of Jollow *et al*. (1974). Briefly, 0.5 ml of 4% sulfosalicylic acid (precipitating agent) was added to 0.5 ml of PMF and centrifuged at 4,000 rpm for 5 minutes. To 0.5 ml of the resulting supernatant, 4.5 ml of Ellman's reagent (0.04 g of DTNB in 100 ml of 0.1 M phosphate buffer, pH 7.4) was added. The absorbance was read at 412 nm against distilled water as blank.

Glutathione S-transferase (GST) was estimated by evaluating the production of a complex formed from the enzymatic conjugation of reduced glutathione with 1-chloro-2,4 nitrobenzene and measuring the absorbance at 340 nm wavelength (Habig *et al*., 1974).

Protein and non-protein thiol contents in samples were determined by reaction with 5,5'-dithiobis (2-nitrobenzoate) as previously described with the initial concentrations of the thiol compounds being in the range of 0.5-1.0 mM (Ellman, 1959).

Protein carbonyls (PC) were measured using the method of Reznick & Packer (1994). The post mitochondrial fraction of samples (1 ml) was added to two test tubes: one containing 4 ml of 2,4-Dinitrophenylhydrazine and 2.5 M hydrochloric acid whereas the other contained 4 ml of hydrochloric acid. The tubes were incubated at room temperature for 1 hour and vortexed every 15 minutes. Thereafter, 5 ml of trichloro acetic acid was added and the tubes

were placed on ice for 10 minutes. Centrifugation was then done for 5 minutes and the supernatants obtained after centrifugation were carefully removed. The precipitates were dissolved in guanidine hydrochloride solution and were left for 10 minutes at 37 °C and vortexed.

Thiobarbituric acid reactive substance (TBARS) was quantified as malondialdehyde (MDA) in the cardiac and renal PMF. The MDA was determined according to the method of Varshney & Kale (1990). Briefly, 0.4 ml of samples and 0.5 ml of 0.75% thiobarbituric acid prepared in 0.2 M HCl were added to 1.6 ml of tris-potassium chloride and 0.5 ml of 30% trichloroacetic acid. The reaction mixture was incubated in the water bath at 80°C for 45 minutes, cooled on ice and centrifuged at 4 000 rpm for 15 minutes. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56 $x10^5$ M/Cm.

Immunohistochemical evaluation

In this study we evaluated the immunohistochemical expressions of angiotensin-converting enzyme (ACE) and podocin in renal tissues of rats as previously described by Oyagbemi *et al*. (2019). The antibodies against ACE and podocin were probed in renal tissues using a 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The renal samples were initially fixed with 10% neutral buffered formalin, embedded in paraffin wax, and sectioned at a thickness of 5 µm. The slides were subsequently dewaxed in xylene (100%) solution for 2 minutes and afterwards, hydration was carried out in different concentrations of ethanol (100%, 90%, and 80%) for 2 minutes each. The hydrated slides were rinsed and put in a PBS buffer tank for 5 minutes. The antigen retrieval was performed with a citrate buffer solution containing 2.1 g of citric acid monohydrate and 14.75 g of trisodium citrate dehydrate adjusted to pH 6.0 in a microwave oven. Endogenous peroxide (H_2O_2 block) was carried out following the manufacturer's instructions as directed on the kit (E-IR-217C). Drops of H_2O_2 were added to cover the sections and incubated in a humidifying chamber at room temperature for 10 minutes. The slides were rinsed afterwards and put back in the PBS tank for 5 minutes. Goat serum (E-1R-R217A) was added onto the slides to prevent nonspecific binding and incubated in the humidifying chamber at room temperature (37oC) for 30 minutes. After 30 minutes of incubation, the tissues were probed with primary antibodies viz-a-viz Angiotensin Converting Enzyme1 Polyclonal Antibody (E-AB-16159: 1:500 Dilution) and podocin antibody (E-AB-16061: 1:150 Dilution) for kidney and were incubated for 2 hours at room temperature. Following incubation, the slides were rinsed with PBS and a secondary antibody labelled (E-1R-R217B) was added, and the slides were incubated in a humidifying chamber at room temperature for 20 minutes. Thereafter, the slides were rinsed and immersed in a PBS tank for 5 minutes. Finally, a few drops of the substrate diaminobenzidine (DAB) were added at room temperature for 10 s; 50 µL of DAB concentrate (E-1R-R217D) + 1 mL DAB solution (E-1R-R217E) in the dark. The reaction was terminated with deionized water and slides were immersed in haematoxylin for 3 s before rinsing with PBS. The slides were placed in 80%, 90%, and 100% of ethanol, and then xylene (100%) for 2 minutes each. Slides were removed, allowed to dry and a DPX mountant was applied. Sections were examined using a digital camera and a Leica software application package version 3.4 light microscope (Leica LAS-EZ®).

Data analysis

The data obtained from this study were analyzed using descriptive statistics and analysis of variance, with the confidence limits set at a 95% confidence interval.

Results

A total weight for the crude extract of 4.846 g was obtained from the extraction of *C. volubile*. Observations in the glycerol group showed significant change in primary haemodynamic parameters (the systolic, diastolic and mean arterial blood pressures), while the extract at all doses and both phases caused a significant ($p<0.05$) decrease of these parameters (Table 1). The methanol leaf extract of *C. volubile* caused a significant lowering of blood pressure in this study. While there was a significant lowering of the heart rate by glycerol, the extract caused an increase. Table 2 showed *C. volubile*-induced alterations on renal enzymatic antioxidant molecules (SOD, GST, GPx, Vitamin C), while Table 3 shows alterations in renal non-enzymatic antioxidant molecules including reduced glutathione (GSH), protein thiol (PSH) and nonprotein thiol (NPSH) compared with the glycerol-treated group. The activities of SOD and GPx were significantly ($p<0.05$) decreased in *C. volubile*-treated rats compared glycerol-treated group. Still, for non-protein thiol, there was a significant decrease in its level. Figure 1 shows the effect of methanol leaf

Table 1: Blood pressure of rats exposed to glycerol and *C. volubile*

Indices	Control	Glycerol	Gly + Cle PreRx100 mg/kg	Gly + Cle PreRx200 mg/kg	Glycerol + Cle PostRx100 mg/kg	Gly + Cle PostRx200 mg/kg
SBP	119.5±6.5	186.2±7.7[a]	145.5±3.1[a,b]	116.8±5.4[b,c]	140.0±14.4[a,b,c]	109.6±4.3[b,e]
DBP	73.8±1.5	124.0±8.0[a]	109.3±2.5[a,b]	79.8±2.8[b]	92.6±4.1[a,b]	76.1±4.5[b]
MABP	88.7±3.2	144.4±14.2[a]	121.0±2.6[a,b]	91.8±3.1[b]	108.3±5.9[a,b]	86.9±4.0[b]
Heart rate	260.5±59.1	214.6±54.3	234.5±24.4	343.2±43.6[a,b,c]	138.7±16.0[a,b,d]	322.5±33.7[b,c,e]

SBP, DBP, MABP = Systolic, Diastolic and Mean Blood pressure respectively in millimetres of mercury. Rx=Treatment. Gly=Glycerol. Cle= *C. volubile*

Table 2: Renal enzymatic antioxidant molecules (SOD, GST, GPx and Vitamin C) of glycerol and *C. volubile*-exposed rats

Parameters	Control	Glycerol	Gly + Cle PreRx100 mg/kg	Gly + Cle PreRx200 mg/kg	Gly + Cle PostRx100 mg/kg	Gly + Cle PostRx200 mg/kg
SOD	43.06±29.9	13.38±39.0*	30.12±28.01[#]	27.01±23.7[#]	26.91±28.7[#]	40.59±23.6[#]
GST	0.03±0.02	0.02±0.02	0.05±0.02	0.03±0.02	0.02±0.00	0.05±0.03
GPx	108.03±39.8	89.49±25.5*	107.94±38.2[#]	125.09±21.4[#]	97.88±48.5[#]	113.42±35.3[#]
Vitamin C	0.24±0.04	0.18±0.02	0.19±0.02	0.19±0.04	0.25±0.1	0.17±0.04

*indicates a significant decrease compared with the control. [#]indicates a significant increase compared with the glycerol group.

SOD= Superoxide dismutase (units/mg protein). GST = Glutathione S-transferase (mmole1 CDNB-GSH complex formed/minutes/mg protein). GPx = Glutathione Peroxidase (units/mg protein). Gly=Glycerol. Cle= *C. volubile*

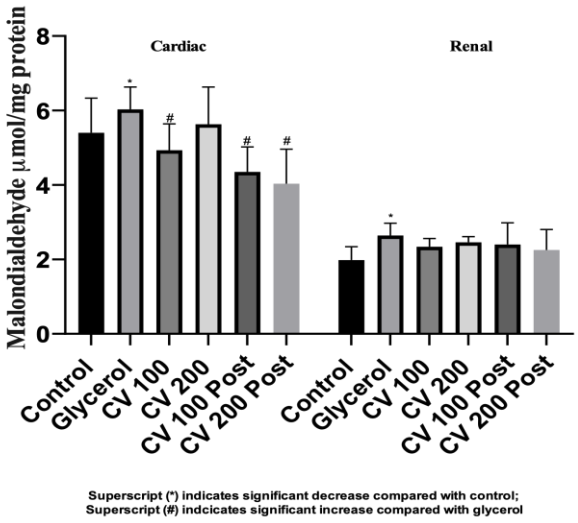


Figure 1: Effect of methanol leaf extract of *Clerodendrum volubile* on cardiac and renal serum indices (MDA). The extract significantly reduced cardiac serum indices pre-(only at 100 mg/kg) and post-treatment at 100 and 200 mg/kg

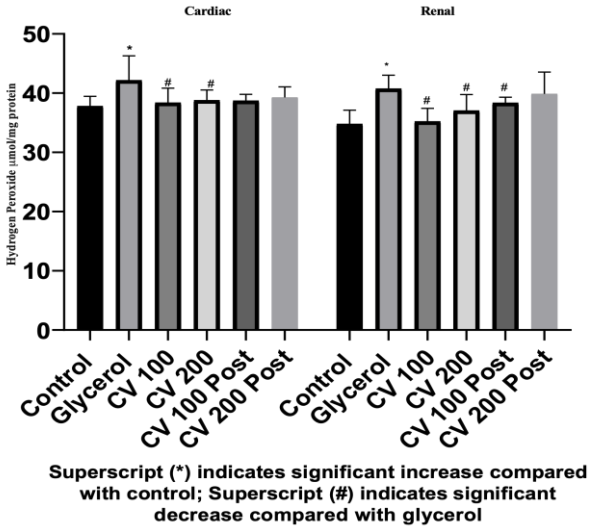


Figure 2: Effect of methanol leaf extract of *Clerodendrum volubile* on cardiac and renal hydrogen peroxide. In this parameter, there was a significant decrease in its level in the cardiac tissues at the pre-treatment phase at the post-treatment phase for the extract-treated groups compared to the glycerol-treated group

extract of *C. volubile* on cardiac and renal serum indices. The extract significantly reduced cardiac serum indices pre-(only at 100 mg/kg) and post-treatment at 100 and 200 mg/kg. Figure 2 shows the effect of methanol leaf extract of *C. volubile* on cardiac and renal hydrogen peroxide. In this parameter, both in cardiac and renal tissues, there was a significant decrease in its level for the extract-treated groups compared to the toxicant group. Figure 3 shows the effect of methanol leaf extract of *C. volubile* on myeloperoxidase (MPO). All the doses in both phases caused a significant decrease in the level of this parameter when compared with the glycerol-treated group. The 200 mg/kg dose at the

pre-treatment phase was particularly effective at causing this decrease. Figure 4 shows the effect of the methanol leaf extract of *C. volubile* on nitric oxide. Furthermore, the 100 mg/kg dose at both phases caused a significant increase in the level of NO, while the 200 mg/kg dose caused an insignificant increase. Plate I shows an immunohistochemical expression of angiotensin-converting enzyme, while Plate II shows the immunohistochemical expression of renal podocin. *C. volubile* caused significant downregulation in the expression of this protein, compared with the glycerol-treated group. The effect of the plant extract on podocin was like that of ACE.

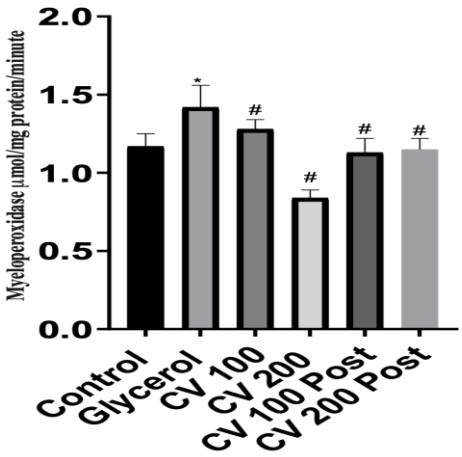
Discussion

Rhabdomyolysis has been established as a clinical syndrome causing muscle injury, myoglobinuria and AKI (Zimmerman & Shen, 2013). Glycerol injection in rats causes rhabdomyolysis with consequent induction of AKI (Reis *et al.*, 2019). In rhabdomyolysis, there may be an injury to the myocyte membrane, resulting in increased intracellular calcium concentrations and initiation of pathological changes. The primary muscle constituent responsible for renal damage in rhabdomyolysis is myoglobin (Zhang, 2012). It has also been shown that there is a link between acute kidney injury and hypertension (Di Nicolo, 2018). Mesangial and endothelial cell injury are considered critical pathophysiologic mechanisms in the renal injury associated with hypertension

Table 3: Renal non-enzymatic antioxidant molecules in glycerol and *C.volubile* exposed rats

Parameters	Control	Glycerol (50%)	Gly + Cle PreRx100 mg/kg	Gly + Cle PreRx200 mg/kg	Gly + Cle PostRx100 mg/kg	Gly + Cle PostRx200 mg/kg
GSH	27.43±7.0	37.12±27.5	41.23 ± 17.7	44.17±22.3	32.13±15.4	37.64±10.03
PSH	80.64±24.1	100.55±19.4	82.46 ± 43.2	84.92±21.6	78.02±18.1	68.92±12.9
NPSH	23.60±4.1	18.40±2.4[a]	18.26 ± 2.6[a]	18.56±2.4[a]	18.49±1.3[a]	18.52±1.3[a]

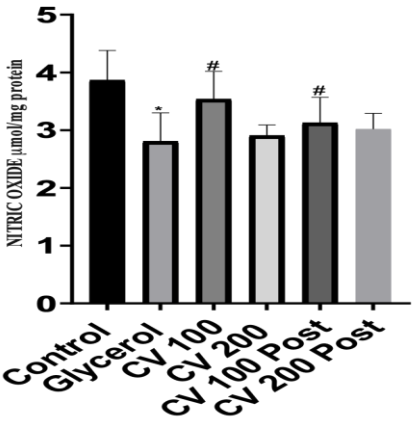
GSH=Reduced glutathione (micromole/mg protein); PSH (PT) = Protein Thiol (nmole/mg protein); NPSH (NPT)=Non-protein thiol (nmole/mg protein)



Superscript (*) indicates a significant increase compared to the control

Superscript (#) indicates a significant decrease compared to glycerol

Figure 3: Effect of methanol leaf extract of *Clerodendrum volubile* on myeloperoxidase (MPO). All the doses in both phases caused a significant decrease in the level of this parameter when compared with the toxicant group. The 200 mg/kg dose at the pre-treatment phase is particularly more effective at causing this decrease



Superscript (*) indicates a significant decrease compared to the control

Superscript (#) indicates a significant increase compared to glycerol

Figure 4: Effect of methanol leaf extract of *Clerodendrum volubile* on nitric oxide. In the control, the 100 mg/kg dose at both phases caused a significant increase in the level of NO while the 200 mg/kg caused an insignificant increase when compared with the toxicant group

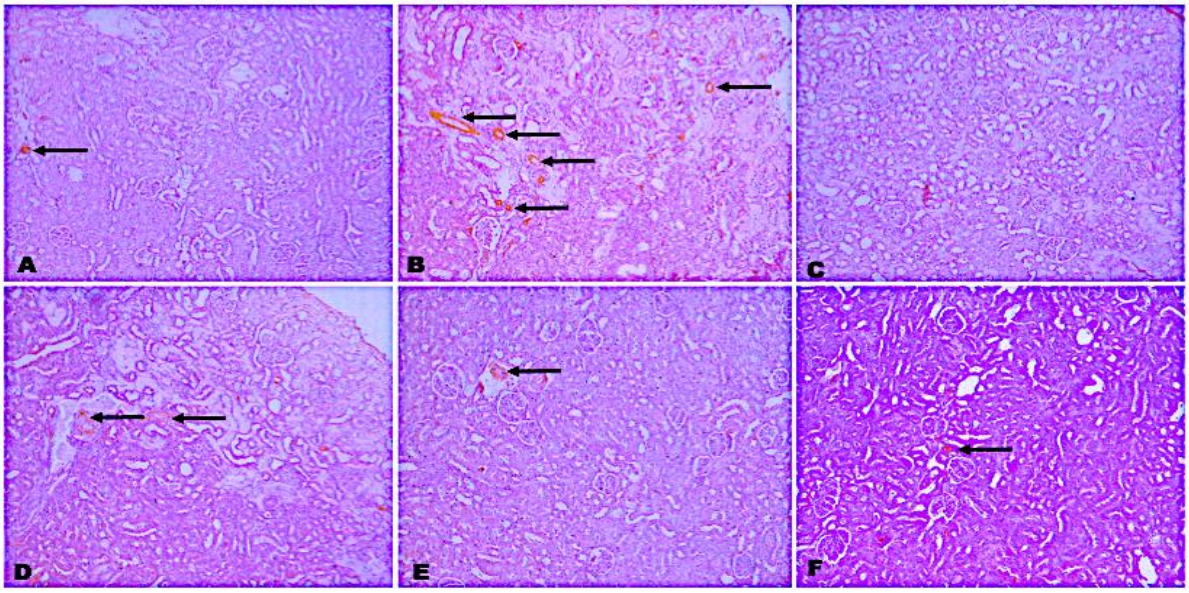


Plate I: The immunohistochemistry of renal angiotensin-converting enzyme (ACE). A (Control), B (Glycerol; 10 mL/kg), C (Glycerol; 10 mL/kg + Pre- Rx *Clerodendrum* 100 mg/kg), D (Glycerol; 10 mL/kg + Pre- Rx-*Clerodendrum* 200 mg/kg), E (Glycerol; 10 mL/kg + post-Rx *Clerodendrum* 100 mg/kg) & F Glycerol; 10 mL/kg + Post Rx-*Clerodendrum* 200 mg/kg. Slides stained with high-definition Haematoxylin. Magnification at X 100

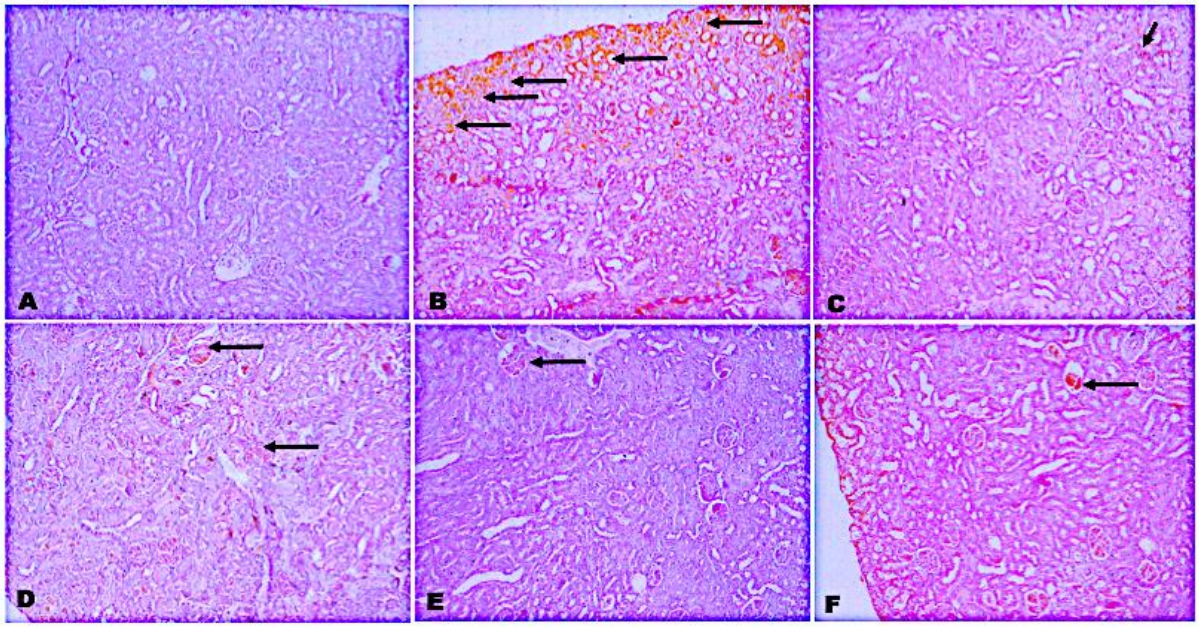


Plate II: The immunohistochemistry of renal podocin. A (Control), B (Glycerol; 10 mL/kg), C (Glycerol; 10 mL/kg + Pre- Rx *Clerodendrum* 100 mg/kg), D (Glycerol; 10 mL/kg + Pre- Rx-*Clerodendrum* 200 mg/kg), E (Glycerol; 10 mL/kg + post-Rx Clerodendrum 100 mg/kg) & F Glycerol; 10 mL/kg + Post Rx-*Clerodendrum* 200 mg/kg. Slides stained with high-definition Haematoxylin. Magnification at X 100

(Basile, 2007). In an earlier report, glycerol-fructose administration resulted in hypertriglyceridemia, hyperinsulinemia, increased vascular sensitivity to 12,13-phorbol dibutyrate and hypertension in rats (Damiano *et al*., 1999). However, a contrasting result was seen in another study where administration of glycerol, did not lead to any change in blood pressure parameters (Drieman *et al*., 1994). The plant extracts of *C. volubile* have been reported to possess high constituent phytochemicals with probable antihypertensive effects (Ajao *et al*., 2018; Okaiyeto *et al*., 2021).

Aggravated production of free radicals and inflammation are centrally involved in the pathogenic

mechanisms leading to AKI (Cebi *et al*., 2016). In this study, myeloperoxidase (MPO), malondialdehyde (MDA) and other markers of oxidative stress were evaluated. MPO is considered to have two main activities: oxidation of halogens to form hypohalous acids and the peroxidase cycle (Khan *et al*., 2014; Siraki, 2021). MPO plays vital roles as part of the innate immune system through the formation of microbicidal reactive oxidants, while modulating the activities of the arterial endothelium, as well as several mechanisms such as the modification of net cellular cholesterol flux and impairment of nitric Oxide (NO)-induced vascular relaxation (Anatoliotakis *et al*., 2013).

The elevation of MDA in the toxicant group suggests glycerol-induced oxidative stress, which is further confirmed by the elevation of hydrogen peroxide (H_2O_2) by glycerol in this study. Contrastingly, the ability of the extract at both doses and in both phases to inhibit the expression of both MDA and H_2O_2 points to the antioxidant property of *C. volubile*. The inhibition of MDA and H_2O_2 which occurred in both cardiac and renal tissues suggests that this plant extract has both nephroprotective and cardioprotective effect. The observations in this study are like the report of Olorundare *et al.* (2020), who reported a cardioprotective effect for *C. volubile* extract in doxorubicin-induced cardiotoxicity via mitigation of oxidative stress and improvement of cardiac histological lesions.

The non-enzymatic and enzymatic renal antioxidants superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also evaluated in this study. The results showed that the methanol leaf extract of the plant caused significant increases in the activities of SOD and GPx when compared with the glycerol group. *C. volubile* is a plant that is rich in phenols which are the most potent natural antioxidants (Adefegha & Oboh, 2016; Olorundare *et al*., 2020). ROS are continuously generated by the mitochondria, but they are kept in check by endogenous cellular antioxidant mechanisms such as SOD and catalase. An imbalance between ROS production and the cellular antioxidant defense system causes oxidative stress (Jena *et al*., 2023). In this study, the observed increase in the activities of SOD and GPx suggests an enhancement of the antioxidant defense systems in the renal tissues of *C. volubile*-treated rats; thus, suggesting a nephroprotective effect for the plant.

Angiotensin-converting enzyme (ACE) is well known for its dual actions in converting inactive Ang I to active Ang II and degradation of active bradykinin, which play an important role in the control of blood pressure (Wong *et al*., 2016). The inhibition of ACE in this study of the plant showed that the plant extract has cardioprotective and nephroprotective properties. Podocin is a critical component of the glomerular filtration barrier that has been reported to decrease in certain diseases of the kidney (Relle *et al*., 2011; Hassan *et al*., 2023). As one of the podocyte proteins, podocin is critical for the maintenance of the structural integrity of the slit diaphragm (Pan *et al*., 2014). In mice, the constitutive absence of podocin leads to rapidly progressive renal disease characterized by mesangiolysis and/or mesangial sclerosis and nephrotic syndrome (Mollet *et al*., 2009). Furthermore, the reduction of podocin levels at the site of the slit diaphragm complex has been reported to be associated with structural and functional derangement in the kidney, whereas podocin is highly expressed in normal glomerular podocytes. Therefore, the significant downregulation of this protein in this study is a pointer to its nephroprotective property of *C. volubile*.

This study concludes that *Clerodendrum volubile* ameliorated glycerol-induced acute renal injury through the inhibition of oxidative stress and modulation of the expressions of angiotensin-converting enzyme and podocin in renal tissues of rats. Therefore, the ingestion of the plant may confer renoprotection in humans and animals.

Acknowledgement

The authors wish to acknowledge Mr OS Agboola for his technical assistance during this study.

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Adefegha SA & Oboh G (2016). Antioxidant and inhibitory properties of *Clerodendrum volubile* leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension. *Journal of Taibah University for Science*, **10**(3): 521–533.

Ajao AA, Oseni OM & Oladipo OT (2018). *Clerodendrum volubile* P. Beauv (Lamiaceae), an underutilized indigenous vegetable of utmost nutritive and pharmacological importance. *Beni-Suef University Journal of Basic and Applied Sciences*, **7**(4): 606–611.

Anatoliotakis N, Deftereos S, Bouras G, Giannopoulos G, Tsounis D, Angelidis C, Kaoukis A &

Stefanadis C (2013). Myeloperoxidase: expressing inflammation and oxidative stress in cardiovascular disease. *Current Topics in Medicinal Chemistry*, **13**(2): 115-138.

Awoyomi OV, Adeoye YD, Oyagbemi AA, Ajibade TO, Asenuga ER, Gbadamosi IT, Ogunpolu BS, Falayi OO, Hassan FO, Omobowale TO, Arojojoye OA, Ola-Davies OE, Saba AB, Adedapo AA, Oguntibeju OO & Yakubu MA (2021). Luteolin mitigates potassium dichromate-induced nephrotoxicity, cardiotoxicity and genotoxicity through modulation of Kim-1/Nrf2 signaling pathways. *Environmental Toxicology*, **36**(11): 2146-2160.

Basile DP (2007). The endothelial cell in ischemic acute kidney injury: implications for acute and chronic function. *Kidney International*, **72**(2): 151–156.

Bonventre JV (2007). Pathophysiology of acute kidney injury: Roles of potential inhibitors of inflammation. *Contributions to* Nephrology, doi.10.1159/000102069.

Cebi G, Yildiz S & Uzun G (2016). The effect of hyperbaric oxygen therapy on rhabdomyolysis-induced myoglobinuric acute renal failure in rats. *Kidney failure*, **38**(9): 1554–1559**.**

Damiano PF, Rosón MI, Armando I, Nowicki S, Dascal E, Cuniberti L, Albornoz LE & de la Riva IJ (1999). Potential role of glycerol leading to rat fructose hypertension. Hypertension, doi.10.1161/01.hyp.34.4.1007.

Dennis JM & Witting PK (2017). Protective Role for Antioxidants in Acute Kidney Disease. *Nutrients*, **9** (7):718.

Di Nicolò P (2018). The dark side of the kidney in cardio-renal syndrome: renal venous hypertension and congestive kidney failure. *Heart Failure Reviews*, **23**(2): 291-302.

Drieman JC, Van Kan FJPM & Thijssen HHW (1994). Regional haemodynamic effects of dopamine and its prodrugs L-dopa and gludopa in the rat and in the glycerol-treated rat as a model for acute renal failure. *British Journal of Pharmacology*, **111**(4): 1117–1122.

Ellman GL (1959) Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82 (1):70-7.

Gornall AG, Bardawill CJ & David MM (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, **177**(2): 751-66.

Habig WH, Pabst MJ & Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249**(22): 7130-7139.

Hassan MM, Hassan MM, Mollick S, Khan MR, Yasmin F, Bairagi AK, Raihan M, Arif SA & Rahman A (2023). A comparative study, prediction and development of chronic kidney disease using machine learning on patients clinical records. *Human-Centric Intelligent Systems*, **3**: 92–104.

Jena AB, Samal RR, Bhol NK & Duttaroy AK (2023). Cellular Red-Ox system in health and disease: The latest update. *Biomedicine & Pharmacotherapy*, doi. 10.1016/j.biopha.2023.114606.

Jollow DJ, Mitchell JR, Zampaglione N & Gillette JR (1974) Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, **11**(3): 151-169.

Kellum JA, Romagnani P & Ashuntantang G (2021). Acute kidney injury. *Nature Reviews Disease Primers*, doi.10.1038/s41572-021-00284-z.

Khan AA, Rahmani AH & Aldebasi YH (2014). Biochemical and pathological studies on peroxidases –an updated review. *Global Journal of Health Sciences*, **6**(5): 87–98.

Makris K & Spanou L (2016). Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. *Clinical Biochemist Reviews*, **37**(2): 85-98.

Masina J, Moolla M, Motara F, Kalla IS & Laher AE (2022). Clinical Profile of Adult Patients Presenting with renal dysfunction to a tertiary hospital emergency department. *Cureus*, doi.10.7759/cureus.21873.

Misra HP & Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, **247**(10): 3170-3175.

Mollet G, Ratelade J, Boyer O, Muda AO, Morisset L, Lavin TA, Kitzis D, Dallman MJ, Bugeon L, Hubner N, Gubler MC, Antignac C & Esquivel EL (2009). Podocin inactivation in mature kidneys causes focal segmental glomerulosclerosis and nephrotic syndrome. *Journal of the American Society of Nephrology*, **20** (10): 2181-2189.

Okaiyeto K, Falade AO & Oguntibeju OO (2021). Traditional uses, nutritional and pharmacological potentials of *Clerodendrum volubile*. *Plants (Basel)*, doi.10.3390/plants10091893.

Olaleye SB, Adaramoye OA, Erigbali PP & Adeniyi OS (2007). Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-

exposed rats. World Journal of Gastroenterology, **13**(38): 5121-5126.

Olorundare OE, Adeneye AA & Akinsola AO (2020). *Clerodendrum volubile* ethanol leaf extract: a potential antidote to doxorubicin-induced cardiotoxicity in rats. *Journal of Toxicology*, doi.10.1155/2020/8859716.

Omóbòwálé TO, Oyagbemi AA, Folasire AM, Ajibade TO, Asenuga ER, Adejumobi OA, Ola-Davies OE, Oyetola O, Gana J, Adedapo AA & Yakubu MA (2018). Ameliorative effect of gallic acid on doxorubicin-induced cardiac dysfunction in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, **29** (1): 19-27.

Oyagbemi AA, Bolaji-Alabi FB, Ajibade TO, Adejumobi OA, Ajani OS, Jarikre TA, Omobowale TO, Ola-Davies OE, Soetan KO, Aro AO, Emikpe BO, Saba AB, Adedapo AA, Oyeyemi MO, Nkadimeng SM, Kayoka-Kabongo PN, McGaw LJ, Oguntibeju OO &Yakubu MA (2020). Novel antihypertensive action of rutin is mediated via inhibition of angiotensin-converting enzyme/mineralocorticoid receptor/angiotensin 2 type 1 receptor (ATR1) signalling pathways in uninephrectomized hypertensive rats. *Journal of Food Biochemistry*, doi.10.1111/jfbc.13534.

Oyagbemi AA, Omobowale TO, Awoyomi OV, Ajibade TO, Falayi OO, Ogunpolu BS, Okotie UJ, Asenuga ER, Adejumobi OA, Hassan FO, Ola-Davies OE, Saba AB, Adedapo AA & Yakubu MA (2019). Cobalt chloride toxicity elicited hypertension and cardiac complications via induction of oxidative stress and upregulation of COX-2/Bax signaling pathway. *Human & Experimental Toxicology*, **38** (5): 519-532.

Pan QR, Ren YL, Zhu JJ, Hu YJ, Zheng JS, Fan H, Xu Y, Wang G & Liu WX (2014). Resveratrol increases nephrin and podocin expression and alleviates renal damage in rats fed a high-fat diet. *Nutrients*, **6**(7): 2619-2631.

Panizo N, Rubio-Navarro A, Amaro-Villalobos JM, Egido J & Moreno JA (2015). Molecular Mechanisms and Novel Therapeutic Approaches to Rhabdomyolysis-Induced Acute Kidney Injury. *Kidney and Blood Pressure Research*, **40**(5):520-32.

Piko N, Bevc S, Hojs R & Ekart R (2023). The Role of Oxidative Stress in Kidney Injury. *Antioxidants (Basel)*, **12**(9): 1772.

Ratliff BB, Abdulmahdi, Pawar R & Wolin MS (2016). Oxidant Mechanisms in Renal Injury and Disease. *Antioxidants & Redox Signaling*, **25**(3): 119-146.

Reis NG, Francescato HDC, de Almeida LF, Silva CGAD, Costa RS & Coimbra TM (2019). Protective effect of calcitriol on rhabdomyolysis-induced acute kidney injury in rats. *Scientific Reports*, doi.10.1038/s41598-019-43564-1.

Relle M, Cash H, Brochhausen C, Strand D, Menke J, Galle PR & Schwarting A (2011). New perspectives on the renal slit diaphragm protein podocin, *Modern Pathology*, 24 (8): 1101-1110.

Reznick AZ & Packer L (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology*, doi.10.1016/s0076-6879(94)33041-7.

Siraki AG (2021). The many roles of myeloperoxidase: From inflammation and immunity to biomarkers, drug metabolism and drug discovery. Redox Biology, 46:102109.

Ugbaja RN, Akinhanmi TF, James AS, Ugwor EI, Babalola AA, Ezenandu EO, Ugbaja VC & Emmanuel EA (2021). Flavonoid-rich fractions from *Clerodendrum volubile* and *Vernonia amygdalina* extenuates arsenic-invoked hepato-renal toxicity via augmentation of the antioxidant system in rats. *Clinical Nutrition Open Science*, doi.10.1016/j.nutos.2020.12.003.

Varshney R & Kale RK (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *International Journal of Radiation Biology*, **58**(5): 733-743.

Wong C, Gerson A, Hooper SR, Matheson M, Lande M, Kupferman J, Furth S, Warady B, Flynn J (2016). Chronic Kidney Disease in Children (CKiD) Study. Effect of elevated blood pressure on quality of life in children with chronic kidney disease. *Pediatric Nephrology*, **31**(7):1129-1136.

Xia Y & Zweier JL (1997). Measurement of myeloperoxidase in leukocyte-containing tissues. *Analytical Biochemistry*, **245**(1): 93-96.

ZarKalai F, Dakhlaoui S, Hammami M, Mkadmini K, Ksouri R & Isoda H. (2022). Phenolic compounds and biological activities of different organs from aerial part of Nitraria retusa (Forssk.) Asch.: effects of solvents. *International Journal of Food Properties*, **25**(1):1524–1538.

Zhang MH (2012). Rhabdomyolysis and its pathogenesis. *World Journal of Emergency Medicine*, doi. 10.1080/1.

Zimmerman JL & Shen MC (2013). Rhabdomyolysis. *Chest*, **144**(3): 1058-1065.