ORIGINAL ARTICLE

Antioxidant effects of *Ximenia Caffra* (*Olacaceae*) methanol leaf extract in cisplatin-induced nephrotoxic rats

*Mutinta Chipuwa[1], Kaampwe Muzandu[2], Lavina Prashar[3], Sergio Muwowo[4]

[1]*The University of Zambia, School of Medicine, Department of Physiological Sciences*
[2]*Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia*
[3]*Department of Physiological Sciences, School of Medicine, Lusaka, Zambia*
[4]*Department of Pathology and Microbiology, University Teaching Hospitals, Lusaka, Zambia*

ABSTRACT

***Background**:* Diseases such as cancer have been associated with oxidative damage by reactive oxygen species. Drug-induced oxidative stress has also been postulated as a mechanism by which drugs like Cisplatin cause toxicities. Plants such as *Ximenia caffra* have been shown to possess secondary metabolites with antioxidant properties that can potentially modulate oxidative stress. Various studies have demonstrated the presence of phenolic compounds with potent antioxidant activity in *Ximenia caffra* extracts. However, these studies only assessed the activity *in vitro*. We investigated the effects of *Ximenia caffra* extract on the antioxidant capacity and markers of oxidative stress *in vivo* using a rat model of Cisplatin-induced oxidative stress-mediated nephrotoxicity.

***Methods**:* A total of 36 male Wistar rats, weighing 150 -180 g, were randomized into six groups, with each group containing six rats. Rats were treated orally with the extract (100 or 200 mg/kg), vitamin C (500 mg/kg) or saline for 10 days. Cisplatin (7.5 mg/kg i.p. single dose) was administered on day eight to induce nephrotoxicity. Animals were euthanized on day 11 and blood and kidney samples were collected for analysis. Total phenolic content, total antioxidant capacity, and malondialdehyde levels were determined using the Folin-Ciocalteu, Ferric Reducing Antioxidant Power, and Thiobarbituric Acid Reactive Species methods, respectively.

***Results**:* The phenolic content of the methanol leaf extract was 128.68 mg GAE/g, representing 12.87% of dry plant extract. Histological changes in rat kidney tissue suggestive of kidney injury accompanied induction with Cisplatin. Cisplatin also produced a decrease in the total antioxidant capacity of Group 3 [(Positive Control); mean kidney 0.337 mM/g, plasma 0.117 mM/g, $p<0.05$] when compared to Group 1 [(Negative Control); mean kidney 0.465 mM/g, plasma 0.177 mM/g, $p<0.05$]; and an elevation in malondialdehyde content of Group 3 [(Positive Control), mean kidney 5.217 nmol/mg, plasma 1.533 nmol/mg, $p<0.05$] when compared to Group 1 [(Negative Control); mean kidney 1.588 nmol/mg, plasma 0.496

***Corresponding author**:
Mutinta Chipuwa
University of Zambia, School of Medicine, Department of Physiological Sciences,
P.O. Box 32379, Lusaka, Zambia;
Email: mutchipuwa@gmail.com

***Keywords**:* Antioxidants; Malondialdehyde, Oxidative stress; Polyphenols; *Ximenia caffra*

This article is available online at: http://www.mjz.co.zm, http://ajol.info/index.php/mjz, doi: https://doi.org/10.55320/mjz.51.2.463

The Medical Journal of Zambia, ISSN 0047-651X, is published by the Zambia Medical Association

nmol/mg, p<0.05]. However, pretreatment with *X. caffra* extracts or Vitamin C before Cisplatin produced an increase in the total antioxidant capacity of Group 4 [(Extract 100 mg/kg + Cisplatin), mean kidney 0.620 mM/g, plasma 0.316 mM/g, p<0.05], Group 5 [(Extract 200 mg/kg + Cisplatin), mean kidney 0.746 mM/g, plasma 0.484 mM/g, p<0.05] and Group 6 [(Vitamin C 500mg/kg + Cisplatin), mean plasma 0.826 mM/g, plasma 0.599 mM/g, p<0.05]; and a reduction in malondialdehyde levels, Group 4 [(Extract 100mg/kg + Cisplatin), mean kidney 3.410 nmol/mg, plasma 0.982 nmol/mg, p<0.05], Group 5 [(Extract 200 mg/kg + Cisplatin), mean kidney 2.833 nmol/mg, plasma 0.751 nmol/mg, p<0.05] and Group 6 [(Vitamin C 500mg/kg + Cisplatin), mean kidney 2.354 nmol/mg, plasma 0.633 nmol/mg, p<0.05].

***Conclusion**:* The observed ability of the extract to increase the antioxidant capacity and reduce malondialdehyde may be attributed to the presence of phytochemicals such as phenolic compounds which have been shown to possess antioxidant activity. Therefore, the methanol leaf extract of *Ximenia caffra* possesses antioxidant activity with the potential to attenuate oxidative stress and confer protection against oxidative stress-mediated diseases and drug toxicities. However, studies of longer duration are needed to further explore the renoprotective potential of the plant extracts. Toxicity studies on *X. caffra* extracts are also required to better guide doses to be used in other *in-vivo* studies.

INTRODUCTION

The occurrence of many chronic conditions specifically degenerative diseases like cancer, cardiovascular diseases, diabetes, Parkinson's disease, Alzheimer's disease, and musculoskeletal disorders has been linked to oxidative damage by reactive oxygen species[1-3]. In addition, oxidative damage has been postulated as one of the mechanisms by which some drugs like Cisplatin cause toxicities[4]. Plants such as *Ximenia caffra* have been shown to possess secondary metabolites with antioxidant properties. Polyphenols, including phenolic acids and flavonoids, possess the ideal structure for antioxidant activity such as free radical scavenging[5]. Medicinal plants with antioxidant activity have also been shown to protect against the damaging effects of oxidative stress, both *in vitro* and *in vivo*[6].

Reactive species including reactive oxygen species (ROS), are highly reactive molecules generated endogenously during normal cellular processes such as oxidative phosphorylation, respiratory burst, or inflammation. They can also be acquired exogenously from radiation, drugs and xenobiotics, environmental pollutants, pesticides, tobacco smoke, and alcohol abuse[2]. At physiological concentrations, reactive species(RS) play an essential role in biological processes like metabolism, immunological defenses, and cell signaling. However, certain pathological states can lead to overproduction of RS and depletion or down-regulation of antioxidant defense mechanisms. This can create a state of imbalance between oxidants and antioxidants in favor of the oxidants, a condition termed oxidative stress, with subsequent damage to biomolecules[2,7].

Lipid peroxidation is one of the major processes that may cause oxidative damage to macromolecules and vital body tissues. Above physiological levels, free radicals can induce the oxidation of lipids, especially polyunsaturated fatty acids, converting them into lipid hydroperoxides. Other by-products of lipid peroxidation include malondialdehyde (MDA), 4-hydroxynonenal, and other aldehydes and epoxides. These by-products of lipid peroxidation are themselves reactive and are capable of interacting and modifying DNA and other macromolecules. This sets off a chain of reactions that generate more reactive species. This ultimately impairs cell membrane function by altering cell membrane integrity, impairing receptor and enzyme function, and, eventually efflux of vital cellular components and cell death by lysis or apoptosis[2,4,8]. As such, MDA and other by-products of lipid peroxidation can be measured as markers of oxidative stress and tissue damage[9].

Induction of oxidative stress is one mechanism responsible for the toxic effects of several drugs in clinical use[4]. For instance, the nephrotoxicity that occurs with Cisplatin therapy has partly been associated with the induction of oxidative stress[8]. Cisplatin (cis-diamminedichloroplatinum (II), CDDP), is used in the treatment of many solid cancers including cervical cancer. However, renal toxicity (nephrotoxicity) is one of the major toxicities limiting cisplatin therapy[6]. The renal proximal tubule is the specific target in cisplatin-induced nephrotoxicity as it is the site most prone to high drug accumulation. The drug is transported into the renal cells through several means including the organic cation transporter (OCT)-2, leading to intracellular accumulation of the drug[8]. Studies have shown that cisplatin increases levels of superoxide anion, hydrogen peroxide, hydroxyl radical, and other free radicals. In addition, depletion of antioxidants such as glutathione, mitochondrial dysfunction, induction of apoptotic pathways, and eventual renal cell death have been shown to accompany cisplatin-induced acute renal failure in rats[8,10]. Antioxidant agents, including natural plant antioxidants, may therefore play a vital role in the modulation of oxidative stress-induced tissue damage and disease[6].

Many of the commercially available synthetic antioxidants for example butylated hydroxyanisole and butylated hydroxytoluene, are associated with serious adverse effects that limit their clinical use compared to those from natural sources. Plants have widely been studied as potential sources of natural antioxidants. Many plant phytochemical compounds, specifically polyphenols, have been shown to possess antioxidant properties as they have an ideal chemical structure for free radical scavenging[5]. Hence, there has been a growing interest in scientific research of medicinal plants as alternative sources of antioxidants that are naturally occurring, potentially with better efficacy and safety profiles[11].

The genus *Ximenia L*. belongs to the Olacaceae family (also classified as Ximeniaceae), a family of shrubs or small to large trees with alternate, simple, petiolate, and exstipulate leaves. It is composed of ten species in the Old and New World tropics[12]. Two of these species, *Ximenia caffra* and *Ximenia americana* have been reported to occur in Zambia[13-15]. *Ximenia caffra*, also commonly known as the large sour plum (English) or Mung'omba (Chitonga), is a widely distributed species that is indigenous to Central, Southern, and Eastern Africa, including Madagascar. It occurs in two varieties, *X. caffra* var. *caffra* and *X. caffra* var. *natalensis*, mainly distinguished by the degree of hairiness of the leaves. *Ximenia caffra* var. *caffra* is more widely distributed and its leaves are hairy compared to var. natalensis whose distribution is limited to certain localities, with hairless leaves[16]. However, the two varieties are commonly not distinguished in literature, by researchers or ethnobotanical users. The species has both nutritive and medicinal value; the edible fruit is rich in vitamins and minerals while different parts of the tree are utilized in traditional medicine. Several studies have verified some of the ethnobotanical medicinal uses of *X. Caffra* such as its antibacterial, anti-gonococcal, anti-inflammatory, and antioxidant activities. Despite the wide use of *X. caffra* in traditional medicine for various ailments and demonstration of antioxidant activity, to the best of our knowledge, no study has assessed the in-vivo antioxidant activity and the potential protective effects of this plant species against oxidative-related tissue damage[17].

The study aimed to investigate the effect of methanol leaf extract of *X. caffra* on the total antioxidant capacity and markers of oxidative stress in the blood and kidney tissue samples of rat models of cisplatin-induced nephrotoxicity. The total phenolic content of the plant extract was also determined as it has often been correlated with high antioxidant activity.

MATERIALS AND METHODS

Research Design

This was an in vivo scientific experimental study in a rat model of oxidative stress-mediated drug-induced nephrotoxicity.

Ethical Considerations

Ethical clearance for the study was obtained from the University of Zambia Biomedical Research Ethics Committee (REF. No. 2487-2022) and authority to conduct the research was granted by the Zambia National Health Research Authority (Ref. No. NRHA0000002/08/04/2022). All the animals were handled according to the National Health Research (US) Guide for Care and Use of Laboratory Animals[18]. In addition, to ensure that the euthanasia of the rats by cervical dislocation was conducted humanely, the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals[19] were adhered to. This included only using rats that weighed below 200g and limiting the procedure to an experienced Veterinary Doctor.

Chemicals and Reagents

Table 1 shows chemical and reagents that were used in the study.

Reagent/Chemical	Source
L-Ascorbic acid (Vitamin C)	Sigma-Aldrich - Germany, United States of America, South Africa
Butylated Hydroxytoluene	
Folin-Ciocalteu reagent	
Gallic acid	
Glacial acetic acid	
Hydrochloric acid (37%)	
Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)	
Methanol	
Potassium chloride	
Sodium acetate trihydrate	
Sodium chloride	
Sodium carbonate anhydrous	
Sodium phosphate dibasic anhydrous	
Sodium phosphate monobasic monohydrate	
2-Thiobarbituric acid (TBA)	
Trichloroacetic acid	
2, 4, 6-Tripyridyl-s-triazine (TPTZ)	
Ethylenediamine tetraacetic acid (EDTA) disodium salt dehydrate	HIMEDIA - India
Ferrous sulfate	Rochelle Chemicals - South Africa
Sodium hydroxide pellets	Titan Biotech Limited - India
Cisplatin 50mg/50ml injection (CISDNA)	Betadrugs Limited - India

Plant Collection

Fresh leaves of *X. caffra* were collected in November 2021 from Sialumya village, Chief Munyumbwe's area in Gwembe District, Southern Province, Zambia (GPS coordinates 16°38'42.4" S 27°46'05.5" E). The plant was taken for identification at the University of Zambia, Department of Biological Sciences. A specimen was also deposited at the University of Zambia Herbarium, accession number 22390 (UZL).

Plant Material Extraction

The fresh leaves were air-dried at room temperature, away from light, for two weeks. They were then ground into a fine powder using an electric blender and stored in air-tight Ziploc bags.

For plant extraction, 360g of dry powder was weighed and macerated in 70% methanol for 72 hours with occasional agitation and then filtered using Whatman No.1 filter paper. The residue was

resuspended in methanol and agitated using a magnetic stirrer for 1 hour and then filtered. The filtrate was evaporated to dryness in a water bath at 40°C. The residue was weighed and stored in the refrigerator until the time of use. The percentage yield was then calculated using the equation:

Percentage Yield = [(M2 - M1)/M] *100

Where M_2 is the mass of the extract and the beaker, M_1 is the mass of the beaker alone and M is the mass of the initial dried sample.

Experimental Animals

Male albino Wistar rats aged 6-8 weeks, weighing between 150 and 180 grams, were obtained from the University of Zambia, Department of Biological Studies. The animals were maintained on a 12-hour light/dark cycle with free access to food (standard rodent pellet diet) and water. They were acclimatized to these conditions for two weeks before the commencement of the experiments.

Experimental Design

The experiment was set up based on the method of Akomolafe et al with some modifications. After two weeks of acclimatization, a total of thirty-six (36) rats were randomized to one of the six (6) study groups consisting of 6 rats in each group. Normal saline, *X. caffra* extract, or Vitamin C was administered to the different treatment groups in a single daily dose (by oral gavage) for 10 days, while Cisplatin was administered as a single dose (intraperitoneally) on day 8 only. Due to a lack of *in vivo* animal studies on *X. caffra*, test doses of 100 mg/kg and 200 mg/kg (body weight) were selected based on the acute toxicity study of *X. americana*, a plant in the same genus as *X. caffra*. According to the study, methanol stem-bark extract of *X. americana* was estimated to have an oral median lethal dose (LD_{50}) greater than 5000 mg/kg in both mice and rats. Treatments were administered as follows:

Group I served as the negative control and received normal saline at 2 ml/kg, orally for 10 consecutive days, and on day 8 only, a single i.p. injection of normal saline was administered 1 hour after the oral saline dose. The negative (normal) control was used as a reference for the other treatment groups. Group II served as the extract-only group and received a 200 mg/kg oral dose of *X. caffra* extract for 10 consecutive days and on day 8 only, a single i.p. injection of normal saline was administered 1 hour after the oral dose of the extract. The purpose of the extract-only group was to test that the high extract dose did not produce any toxic effect that could affect the results. Group III served as the positive control group and received normal saline orally for 10 consecutive days and on day 8 only, a single dose of cisplatin (7.5 mg/kg, i.p.) was administered 1 hr after the oral saline dose. The positive control was used to test the effects of cisplatin in the absence of an intervention. Group IV served as the test group and received a 100 mg/kg oral dose of *X. caffra* extract for 10 consecutive days and on day 8 only, a single dose of cisplatin (7.5 mg/kg, i.p.) was administered 1 hr after the oral extract dose. Group V also served as the test group and received a 200 mg/kg oral dose of *X. caffra* extract for 10 consecutive days and on day 8 only, a single dose of cisplatin (7.5 mg/kg[6,10], i.p.) was administered 1 hr after the oral extract dose. Group VI served as the standard and received ascorbic acid (Vitamin C), 500 mg/kg[21] orally for 10 days, and on day 8 only, a single dose of cisplatin (7.5 mg/kg, i.p.) was administered 1 hr after the oral Vitamin C dose.

Preparation of Biological Samples

On the 11th day, 72 hours after Cisplatin induction and overnight fast, all the animals were sacrificed by cervical dislocation. Blood samples were then rapidly collected by direct heart puncture into ethylenediaminetetraacetic acid (EDTA) containers, and centrifuged at 800 x g for 10 minutes to separate the plasma. The plasma was then decanted into plain sample containers and stored in the refrigerator until analysis. The rat's kidneys were excised and rinsed in ice-cold saline (0.9% w/v). Following the isolation of both kidneys, a longitudinal section of the right kidney (only for uniformity purposes) from each animal was fixed in 10% formalin for

histopathological examination. The remaining kidney tissue was weighed and homogenized in phosphate-buffered saline (pH 7.4) with (0.1g tissue/ml of buffer) using mortar and pestle as a homogenizer. The homogenates were then centrifuged at 4000 x g to obtain a clear supernatant for the assays[6,22].

Determination of Total Phenolic Content of *X. caffra* leaf extract

The TPC was measured using the Folin-Ciocalteu method[23] with some modifications[24, 25]. Briefly, 0.5 mL of sample was mixed with 0.5 mL of 10% (v/v) Folin-Ciocalteu's reagent and incubated at room temperature, in the dark, for 5 minutes. Then 3 mL of distilled water was added, followed immediately by 2 mL of 20% sodium carbonate (anhydrous). The mixture was then incubated for 2 hours at room temperature in the dark. The absorbance of the solutions was measured with a UV-visible spectrophotometer at 760 nm wavelength against a blank. The standard gallic acid curve was then constructed based on the prepared gallic acid standard solutions (0 to 100 µg/mL). The Phenolic content of the extract was then determined from the GA standard curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample, as follows:

Total phenolic content = GAE x V/ m

Where GAE is the concentration of gallic acid estimated from the standard curve (mg/mL), V is the final volume of the extract (mL) and m is the weight of pure plant extract (g).

Determination of Total Antioxidant Capacity

The TAC of the plasma and kidney tissues was estimated using the Ferric Reducing Antioxidant Power (FRAP) assay based on the methods of Benzie and Strain[26] with slight modifications[27]. The FRAP reagent was prepared using 0.3 mol/L (300 mmol/L) acetate buffer (pH 3.6), 10 mmol/L TPTZ solution in 40 mmol/L hydrochloric acid (HCl) and 20 mmol/L iron (III) chloride solution (FeCl3.6H2O) in a proportion of 10:1:1 (v/v), respectively. Aqueous solution of Fe (II) at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mmol/L were used to construct a standard curve. Initially, 3000 µL (3 ml) of freshly prepared FRAP reagent was warmed at 37°C and a blank reagent reading was taken at 593 nm. Then, 100 µL of the test sample and 300 µL of distilled water were added to the FRAP reagent (3000 µL). This gave a sample dilution in the total volume of the reaction mixture to be 1/34. The FRAP reagent, sample, and water mixture were then incubated at 37°C for 4 minutes. After that, absorbance was read at 593 nm wavelength. The change in absorbance between the final reading selected and that of the blank reagent was calculated for each sample and related to the absorbance of the Fe (II) standard solution tested in parallel. A standard curve of Fe (II) solution was constructed and used to estimate FRAP values of the test samples.

Determination of Malondialdehyde Levels

The plasma and kidney tissue concentrations of MDA were determined using the Thiobarbituric Acid Reactive Species (TBARS) assay. The TBARS adducts yield a red-pink color that can be measured spectrophotometrically at 532 nm[28, 29]. The procedure was carried out based on the modified methods of Esteribauer and Cheeseman[30] and Ho et al[22] as follows: 750 µL of the sample (plasma or kidney tissue homogenate) was mixed with 750 µL of phosphate-buffered saline (pH 9.4) and 75 µL of 0.88% (w/v) butylated hydroxytoluene (BHT). BHT was added to prevent lipid oxidation during the assay. Then 3 ml of 30% Trichloroacetic acid (TCA) was added to precipitate protein in the mixture. The mixture was then incubated on ice for 2 hours and pelleted. An aliquot of the supernatant was mixed with 22.5 µL 0.1 M Ethylenediaminetetraacetic acid (EDTA) and 750 µL of 1% thiobarbituric acid (TBA) in 0.05 M sodium hydroxide and incubated in a boiling water bath (100 °C) for 15 minutes. After cooling the mixture to room temperature, the absorbance of the samples was read at 532 nm (A532) and then at 600 nm (A600).

Malondialdehyde equivalents were then calculated using the method of Heath and Packerand the results were expressed as nmol MDA per mg sample as follows;

MDA equivalents (nmol)/mg = [(A532 – A600)/155]103

Where A532 is the maximum absorbance of the TBA-MDA complex, A600 is the correction for non-specific turbidity, and 155 is the molar Extinction Coefficient TBA-MDA complex (mM-1cm-1).

Data Analysis

Results were expressed as Mean ± Standard Error of the Mean (SEM). Differences between group means were estimated using One-way Analysis of Variance (ANOVA) followed by post hoc tests for multiple comparisons. The Tukey's Honest Significant Difference (Tukey's HDS) post hoc test was used for TAC, while the Games-Howell post hoc test was used for MDA levels, $p < 0.05$ were considered statistically significant; SPSS version 23.0 was used for analysis.

RESULTS

Percentage Yield and Total Phenolic Content of *X. caffra* Leaf Extract

The percentage yield of the plant sample after maceration in 70% methanol and concentration to dryness was 33.05%, with a weight yield of 112.38g of dry extract powder. The TPC determined using the Folin-Ciocalteu method was 128.68 mg GAE/g of plant sample, representing a percentage phenolic content of 12.87% of dry plant extract as shown below.

The mean absorbance readings of Gallic acid standard solutions and *X. caffra* extract following the Folin-Ciocalteu assay for phenolic content are shown in Table 2. The mean absorbance of the extract after diluting by a factor of 4 was 0.252 (± 0.003).

Table 2: The Mean Absorbance and Standard Error of Gallic Acid and *X. caffra*

Concentration	Mean Absorbance (SE)
Gallic Acid (µg/ml)	
0	0.010 (0.001)
5	0.067 (0.009)
10	0.098 (0.002)
20	0.184 (0.006)
30	0.248 (0.002)
40	0.326 (0.005)
50	0.385 (0.004)
60	0.462 (0.003)
80	0.546 (0.012)
100	0.706 (0.011)
***Ximenia Caffra* (1mg/ml)**	0.252 (0.003)

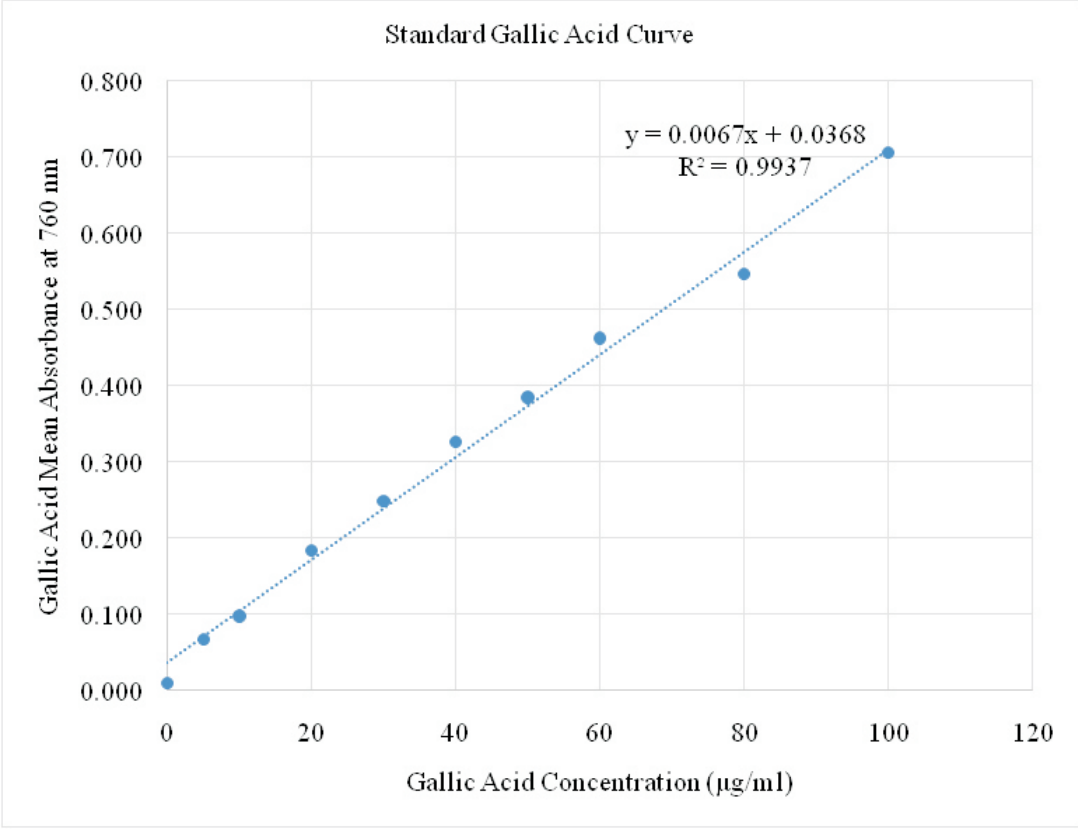

Figure 1: Standard Gallic Acid for Total Phenolic Content Determination

A plot of the gallic acid standard concentrations and the corresponding mean absorbance yielded a standard curve with the equation: $y = 0.0067x + 0.0368$; $R^2 = 0.9937$ (Fig. 1). Using this equation and mean absorbance of 0.252 (Table 2), the phenolic content of *X. caffra* expressed as gallic acid equivalent (GAE) was calculated as follows:

$$y = 0.0067x + 0.0368$$

Where, y is the absorbance and x is the gallic acid concentration (µg/ml)

*X. caffra*GAE = (0.252 -0.0368)/0.0067 (µg/ml)
= 32.17 µg/mL
= 0.03217 mg/mL GAE

Then total phenolic content was estimated as follows:

Total phenolic content = ((GAE*V)/ m)*DF

Where;
GAE = 0.03217 mg/mL GAE
V – Final volume of the extract (mL) = 250 ml
m – Weight of pure plant extract (g) = 0.250 g
DF – Dilution Factor for the extract = 4

X. caffra Total Phenolic Content (TPC) = ((0.03217 mg/ml * 250ml)/*0.250g)*4
= 128.68 mg GAE/g sample

Effect of Cisplatin on Rat Kidney

Figure 2 shows normal renal tubules and glomeruli, with no histological evidence of kidney injury, of kidney samples from rats that were not induced with cisplatin. In contrast, administration of a single dose of cisplatin (7.5 mg/mg) resulted in histological changes and varying degrees of kidney tissue injury in the induced groups. The kidney injuries included acute interstitial injury, acute tubular necrosis, and glomerulosclerosis (Fig. 3).

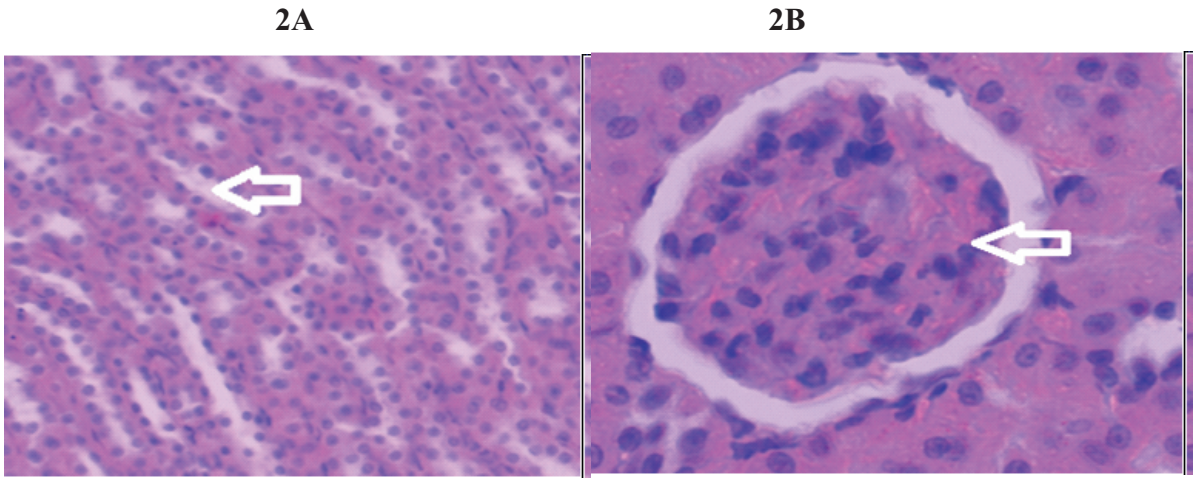

Figure 2: Normal renal tubules and normal glomerulus of kidneys from rats not induced with Cisplatin. Panel **2A** shows a normal renal tubule with well-organized cellular boundary from rats treated with *Ximenia caffra* extract only. Panel **2B** shows normal glomerulus from rats treated with normal saline only (Negative Control)**.**

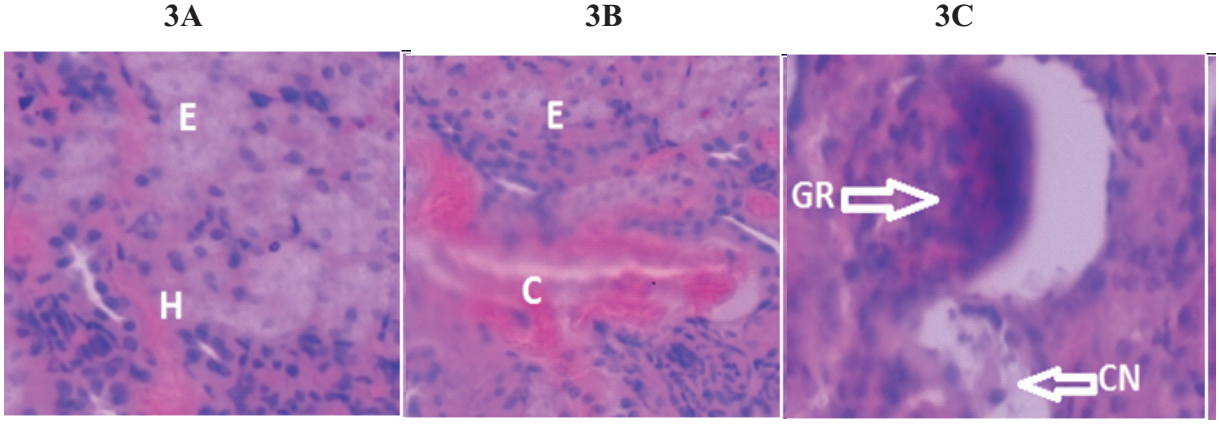

Figure 3: Nephrotoxic effects of Cisplatin on interstitium, renal tubules, and glomerulus of kidneys from induced rats. Panel **3A** shows acute interstitial injury with edema (E) of renal tubules and interstitial Hemorrhage (H). Panel **3B** shows acute interstitial injury with edema (E) of tubules and congestion (C) of renal arteries. Panel **3C** shows glomerulosclerosis with glomerular retraction (GR) and cell necrosis (CN) of renal tubule cells.

Effect of *X. caffra* on Total Antioxidant Capacity

To determine the total antioxidant capacity of the plasma and kidney tissue samples, a standard curve of Iron (Fe (II)) at concentrations between 0.1 and 1.0 mmol/L was constructed using Microsoft Excel 2013. The standard curve was used to calculate ferric reducing antioxidant capacity (FRAP) values, as a measure of the total antioxidant capacity of test samples. The mean total antioxidant capacity of the groups was used for statistical analysis using One-way ANOVA followed by the Tukey post hoc test for multiple comparisons.

A plot of the standard Fe (II) concentrations against mean absorbance yielded a standard curve with the

equation: $y = 0.7509x + 0.0237$; $R2 = 0.9777$. Using the equation, the FRAP values of plasma and kidney tissue samples were calculated using the mean absorbance of each sample. In the equation, y= mean absorbance of the sample and x= total antioxidant capacity in FRAP values that were expressed as mM Fe (II)/g protein.

The results (Table 3 and Fig. 4) revealed a significant reduction in the mean TAC of both blood and kidney samples from rats that received a single dose of Cisplatin-only (positive control group) when compared to the negative control group ($p<0.05$). On the other hand, samples of blood and kidney tissues from rats treated with either *X. caffra* extract (100mg/kg and 200mg/kg) or Vitamin C before cisplatin-induction showed an increase in the TAC when compared to the negative and positive controls ($p<0.05$). The lowest mean TAC was observed in the Cisplatin-only group (Positive Control, mean 0.337 mM/g protein for kidney and 0.117 mM/g protein for blood). Rats that had received the standard antioxidant, Vitamin C showed the highest increase in TAC, higher than the plant extract administered at 200 mg/kg. When comparing the plant extract treated groups, the TAC of rats that received the higher dose of 200 mg/kg had higher mean TAC than those from the low dose of 100 mg/kg. It was also observed that administration of the extract only without induction did not result in a significant increase in TAC when compared to the negative controls that only received saline.

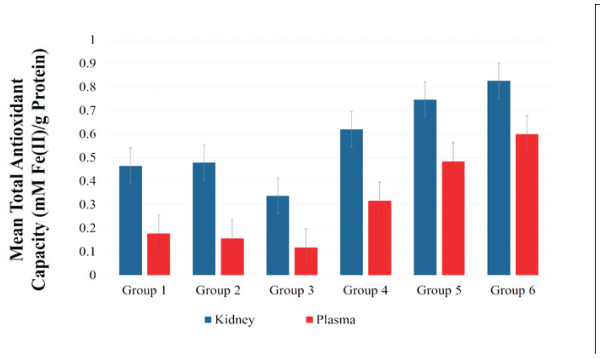

Figure 4: The Total Antioxidant Capacity (mM Fe (II)/g protein) of the kidney and blood (plasma) samples of the different treatment groups (n=6): Group 1: Negative Control (Normal saline), Group 2: Extract only (200mg/kg), Group 3: Positive Control (Cisplatin only); Group 4: Extract 100 mg/kg + Cisplatin, Group 5: Extract 200 mg/kg + Cisplatin, Group 6: Vitamin C 500 mg/kg + Cisplatin. Differences in means were analyzed using a One-way analysis of Variance, followed by the Tukey HSD Post Hoc test, $p<0.05$ was considered statistically significant.

Table 3: Total Antioxidant Capacity (mM Fe (II)/g protein) of Kidney and Plasma Tissue

Group	Treatments	Kidney	Plasma
1	Negative Control (Saline)	$0.465^{c} \pm 0.009$	$0.177^{c} \pm 0.009$
2	Extract Only	$0.479^{c} \pm 0.010$	$0.156^{c} \pm 0.007$
3	Positive Control (Cisplatin)	$0.337^{b} \pm 0.009$	$0.117^{b} \pm 0.006$
4	100 mg/kg extract + Cisplatin	$0.620^{bc} \pm 0.011$	$0.316^{bc} \pm 0.008$
5	200 mg/kg extract + Cisplatin	$0.746^{bc} \pm 0.016$	$0.484^{bc} \pm 0.007$
6	Vitamin C + Cisplatin	$0.826^{bc} \pm 0.016$	$0.599^{bc} \pm 0.011$

Data represent mean (mM Fe (II)/g protein) ± standard error (n=6) of the treatment groups: Group 1: Negative Control (Normal saline), Group 2: Extract only (200mg/kg), Group 3: Positive Control (Cisplatin only); Group 4: Extract 100 mg/kg + Cisplatin, Group 5: Extract 200 mg/kg + Cisplatin, Group 6: Vitamin C 500 mg/kg + Cisplatin. Differences in means were analyzed using One-way analysis of Variance, followed by the Tukey HSD Post Hoc test. A significant difference ($p<0.05$) from the Negative Control (Group 1) and the Positive Control (Group 3) is shown by ^{b} and ^{c} respectively.

Effect of *X. caffra* on Malondialdehyde Levels

The results in Table 4 and Fig. 5 show that the administration of a single dose of Cisplatin increased the MDA levels in blood and kidney tissues of all induced rats as compared to those of the negative controls ($p<0.05$). The highest increase in malondialdehyde content was observed in Group 3 [(Positive Control), mean kidney 5.217 nmol/mg, plasma 1.533 nmol/mg, $p<0.05$] when compared to Group 1 [(Negative Control); mean kidney 1.588 nmol/mg, plasma 0.496 nmol/mg, $p<0.05$]. On the other hand, samples of blood and kidney tissues from rats treated with either *X. caffra* extract (100mg/kg and 200mg/kg) or Vitamin C before cisplatin-induction showed a decline and a reduction in malondialdehyde levels, Group 4 [(Extract 100mg/kg + Cisplatin), mean kidney 3.410 nmol/mg, plasma 0.982 nmol/mg, $p<0.05$], Group 5 [(Extract 200 mg/kg + Cisplatin), mean kidney 2.833 nmol/mg, plasma 0.751 nmol/mg, $p<0.05$] and Group 6 [(Vitamin C 500mg/kg + Cisplatin), mean kidney 2.354 nmol/mg, plasma 0.633 nmol/mg, $p<0.05$] when compared to the positive control group ($p<0.05$). Rats that had received the standard antioxidant, Vitamin C showed a lower MDA content than the plant extract administered at 200 mg/kg. When comparing the plant extract-treated groups, the MDA levels of rats that received the higher dose of 200 mg/kg had lower mean MDA than those that had received the extract at 100 mg/kg. However, the MDA levels of both the plant extract and Vitamin C-treated groups were still higher than the negative control group ($p<0.05$).

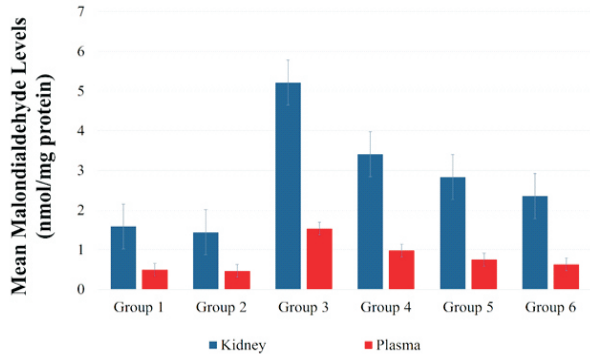

***Figure* 5**: Mean MDA levels of kidney and Plasma samples of treatment groups (n=6): Group 1: Negative Control (Normal saline), Group 2: Extract only (200mg/kg), Group 3: Positive Control (Cisplatin only); Group 4: Extract 100 mg/kg + Cisplatin, Group 5: Extract 200 mg/kg + Cisplatin, Group 6: Vitamin C 500 mg/kg + Cisplatin. Differences in means were analyzed using a One-way analysis of Variance with the Welch Robust Test for Equality of Meansfollowed by the Games-Howell Post Hoc test, $p<0.05$ was considered statistically significant.

Table 4: Mean Malondialdehyde Levels (nmol/mg protein) in Kidney and Plasma Samples

Group	Treatments	Kidney	Plasma
1	Negative Control (Saline)	$1.588^{c} \pm 0.119$	$0.496^{c} \pm 0.029$
2	Extract Only	$1.443^{c} \pm 0.095$	$0.466^{c} \pm 0.032$
3	Positive Control (Cisplatin)	$5.217^{b} \pm 0.206$	$1.533^{b} \pm 0.014$
4	100 mg/kg extract + Cisplatin	$3.410^{bc} \pm 0.109$	$0.982^{bc} \pm 0.027$
5	200 mg/kg extract + Cisplatin	$2.833^{bc} \pm 0.057$	$0.751^{bc} \pm 0.011$
6	Vitamin C + Cisplatin	$2.354^{bc} \pm 0.101$	$0.633^{bc} \pm 0.008$

Data represent mean MDA (mM/g protein) ± standard error (n=6) of the treatment groups: Group 1: Negative Control (Normal saline), Group 2: Extract only (200mg/kg), Group 3: Positive Control (Cisplatin only); Group 4: Extract 100 mg/kg + Cisplatin, Group 5: Extract 200 mg/kg + Cisplatin, Group 6: Vitamin C 500 mg/kg + Cisplatin. Differences in means were analyzed using a One-way analysis of Variance with the Welch Robust Test for Equality of Means followed by the Games-Howell Post Hoc test. A significant difference ($p<0.05$) from the Negative Control (Group 1) or the Positive Control (Group 3) is shown by ^{b} and ^{c} respectively.

DISCUSSION

In assessing the effectiveness of the rat model of cisplatin-induced nephrotoxicity in this study, histopathological examination of sections of the rat kidney revealed the presence of varying degrees of renal injury that included acute tubular necrosis, acute interstitial injury and glomerulosclerosis in the cisplatin-induced groups. This was in contrast to the negative control and *X. caffra*-only groups that showed normal histology with no evidence of renal injury. The observed renal injuries in the cisplatin-induced groups were suggestive of renal injury and nephrotoxicity[8]. The findings of the current study are consistent with those of Akomolafe et al[6] who reported severe and generalized tubular epithelial cell necrosis associated with diffuse tubular lumina in rat kidneys following a 7.5 mg/kg i.p. dose of cisplatin. The histological changes in the kidney tissue similar to those reported by other studies, suggest that the model of nephrotoxicity had been attained in this study[10].

The study demonstrated the presence of phenolic compounds in the leaves of *X. caffra* as evidenced by the high total phenolic content (12.87% of dry plant extract) determined by the Folin-Ciocalteu assay. Phenolic compounds amounting to 4.5% of the dry plant extract are considered to be significantly high and likely to possess antioxidant activity[32]. The findings of this study are in line with Zhen et al[25] who reported high TPC in the 70% methanol leaf extract of *X. caffra* of 261.87 ± 7.11 mg GAE/g, representing about 26% of the dry plant extract. Another study, however, reported a much lower phenolic content of 29.7 ± 1.45 mg GAE/g (about 3% of dry plant extract) in the leaves of *X. caffra* where 100% methanol was used as the solvent for extraction[24]. Apart from differences in the solvent used for plant extraction and the extraction method, one other factor that can explain the variations in the TPC of the same plant species is the difference in geographical location and climate from which the plant samples are obtained[33].

The effect of *X. caffra* on the TAC was evaluated based on the ability of components in the plasma and kidney samples to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The current study showed a significant reduction in the TAC of both plasma and kidney tissue samples in rats that received Cisplatin alone. In contrast, treatment with *X. caffra* extract along with Cisplatin increased the TAC of the rats when compared to the negative and positive control groups. It was also observed that administration of *X. caffra* extract alone did not result in higher total antioxidant capacity when compared to the groups that had received *X. caffra* extract and induced with cisplatin. This indicated that in the absence of stress, the extract did not alter the physiological levels of antioxidants The effects of *X. caffra* extract on the TAC were also seen to be in a dose-dependent manner, with rats that had received the higher extract dose of 200 mg/kg having higher mean values compared to those that had received the lower extract dose of 100 mg/kg. However, the observed effect of *X. caffra* extract on the TAC was lower than that of the reference antioxidant, Vitamin C.

The findings of the study are similar to the results of Verma et al[34] who reported a reduction in the total antioxidant status of rats induced with cisplatin (12 mg/kg) without any other treatment. Pre- and post-treatment with *Calendula officinalis* floral extracts along with cisplatin showed a significant increase in the total antioxidant status in the blood and renal tissue samples of rats. Similarly, administration of *Calendula officinalis* extracts alone (without cisplatin induction) did not result in higher total antioxidant capacity when compared to those that received the extracts along with cisplatin. The study also showed that the increase in the total antioxidant status of rats in extract-treated groups was still lower than that of the negative control group. These results are contrary to the current study where the administration of *X. caffra* extract along with cisplatin resulted in significantly higher TAC when compared to the normal control.Other studies have also reported similar findings[35]. The observed variations may be attributed to the differences in the

Cisplatin protocols as studies have shown that Cisplatin models of nephrotoxicity may differ with the dose and duration of treatment[10]. In addition to differences in the disease models and setup of the experiment, the plant species under comparison are different with different phytochemical contents. In comparison to other studies on the same plant species, several researchers have reported high total antioxidant capacity of different extracts of *X. caffra* such as its strong reducing power when compared to standard controls like vitamin C[25, 36, 37]. However, all these studies that reported this good antioxidant activity of *X. caffra* extracts only ended at assessing the activity using *in vitro* assays.

The FRAP assay estimates the capacity of antioxidants present in a given sample to act as a reductant in a redox reaction, in this case reducing Fe^{3+} to Fe^{2+} by donating an electron[38]. The ability of *X. caffra* to increase the TAC in the induced rats implies the presence of compounds in the extract that possess reducing properties that exert their effect by donating electron(s) or hydrogen atoms. Chelation of redox-active metal ions and reduction of other ROS are some of the mechanisms by which plant phytochemicals, such as phenolic compounds act to attenuate oxidative stress and associated damage[2, 5]. The high presence of total phenolic content already established in this study may be responsible for the activity as phenolic compounds have demonstrated the ability to reduce free radicals[24]. Therefore, the observed ability of *X. caffra* to increase the total antioxidant capacity in cisplatin-induced groups reflects its capability to modulate oxidative stress by acting as a reducing agent, resulting in the interruption of the free radical chain.

Administration of a single dose of cisplatin resulted in a significant increase in the kidney tissue and plasma malondialdehyde (MDA) levels in all the treatment groups that had received the drug (cisplatin) when compared to the negative control group and the group that only received *X. caffra*. However, a decrease in the MDA levels of kidney tissue and plasma samples was observed in the treatment groups that had received both *X. caffra* extract and cisplatin injection when compared to the positive control. The reduction in the MDA levels of the groups that had received both the extract and cisplatin was seen to be in a dose-dependent manner, with rats that received doses of 200 mg/kg of extract having lower mean values compared to those that received doses of 100 mg/kg. When compared to the standard antioxidant, the extent to which *X. caffra* extract decreased the MDA levels was not superior to that of Vitamin C at the experimental dosages. It was also shown that administration of the extract alone, without cisplatin, did not significantly affect MDA levels when compared to the negative control. This indicates that the plant extract did not induce oxidative stress which could have increased the levels of malondialdehyde.

The results from this study are similar to the findings of Akomolafe et al[6] who reported marked elevation in rat kidney and plasma MDA content after a dose of cisplatin as compared to the negative control group. The study also reported that pretreatment of rats with gallic acid and tannic acid, before cisplatin administration resulted in a significant reduction in MDA content when compared with the induced (positive) control group. Another study also reported an increase in MDA levels after cisplatin induction while treatment with curcumin resulted in a decline in MDA content[39]. In contrast, the results of Hosseinian et al[40] showed no significant increase in kidney tissue MDA concentrations after a dose of cisplatin (6 mg/kg) in rats when compared to the negative control. In addition, administration of a plant extract (*Nigella sativa*) before and after cisplatin-induction did not significantly reduce MDA levels when compared to the cisplatin-only group. These variations could be due to differences in dosage and duration of cisplatin induction[10], and the fact that this involved a different plant species than the one under study.

Malondialdehyde, one of the by-products of lipid peroxidation is often used as an analytical marker of lipid peroxidation and oxidative stress[28, 41]. Cisplatin has been shown to increase ROS such as hydrogen peroxide leading to the oxidation of lipids, particularly the polyunsaturated fatty acids, subsequently causing damage to biomolecules[8]. This observation was made by other studies where an increase in hydrogen peroxide concentrations and depletion of antioxidant enzymes resulted in elevated MDA levels due to lipid peroxidation[6]. The increase in MDA levels after a dose of cisplatin shown in the current study suggests that the drug had induced lipid peroxidation leading to nephrotoxicity[10]. However, the reduction in MDA levels in rats that had received *X. caffra* extract and Cisplatin, suggests improvement in the oxidative status and reduction in lipid peroxidation. This implies that the plant extract contains constituents with antioxidant properties that are capable of modulating ROS, thereby improving oxidative status and reducing lipid peroxidation and associated damage to the rat kidneys.

In understanding the relationship between TPC and the observed antioxidant activity, the activity of the extract was compared with that of the standard antioxidant, Vitamin C. The results of the study showed that rats that had received *X. caffra* with cisplatin or vitamin C with cisplatin had significantly higher total antioxidant capacity values and lower MDA levels when compared to the cisplatin-only group. However, rats that had received vitamin C had significantly higher total antioxidant values and lower MDA levels than the extract-treated groups. This implies that the antioxidant activity of the extract, despite the high total phenolic content reported, was not comparable to that of vitamin C at the experimental dosages. In contrast to the findings of this study, other studies that have investigated the antioxidant activity of *X. caffra* have reported that the plant extracts had exhibited antioxidant activities that were comparable and in some cases superior to those of standards like vitamin C, Quercetin and Trolox, despite reporting much lower total phenolic content than this study[24, 25, 42]. However, this comparison is limited by the fact that all these studies were *in vitro* and good *in vitro* activity may not necessarily translate into good *in vivo* activity, putting into consideration the effects of pharmacokinetics and pharmacodynamics on biological activity in a live model. The observed differences can also be explained by the fact that the dose for Vitamin C was optimized while the two doses for the plant extract were not. The dose-dependent increase in the extract activity between the lower and higher doses suggests that at optimized doses, the plant has the potential to show better activity. However, toxicity studies are needed to better guide the selection of optimized doses for *X. caffra*.

Increasing the total antioxidant capacity and decreasing MDA levels reflects on the antioxidant activity of *X. caffra* such as the power to reduce ROS to more stable forms. This mechanism can attenuate oxidative stress and prevent tissue damage and associated diseases. The demonstrated *in vivo* activity shows the plant's potential as a source of natural antioxidants. The established high total phenolic content also indicates that in addition to antioxidant activity, the plant might possess other activities such as antimicrobial, anti-inflammatory, and antiproliferative properties[17]. This study also justifies some of the ethnobotanical uses of the plant.

LIMITATIONS

The study was limited by the absence of preliminary acute toxicity studies of *Ximenia caffra* extracts which could have given a more accurate estimate of test doses to use in the study. With the 200 mg/kg extract dose showing higher antioxidant activity than the 100 mg/kg dose, the availability of acute studies would guide future studies to use optimized doses that could show an even better activity. The

findings of this study showed that the plant was not toxic at doses of 200 mg/kg in rats as no mortality was recorded. However, the study was of a short duration and other effects might not have been apparent at the end of the study. Therefore, there is need also for *in vivo* antioxidant studies of longer duration to understand the renoprotective potential of the plant extracts.

CONCLUSION

The study revealed that a single dose of cisplatin decreased the total antioxidant capacity and increased malondialdehyde (MDA) levels of induced rats, suggestive of increased oxidative stress, lipid peroxidation, and consequently nephrotoxicity. The study also showed that administration of the methanol leaf extract of *X. caffra* increased the total antioxidant capacity and reduced MDA levels in both the kidney tissue and plasma of cisplatin-induced rats in a dose-dependent manner. The observed ability of the extract to increase the antioxidant capacity and reduce malondialdehyde may be attributed to the presence of phytochemicals such as phenolic compounds which have been shown to possess antioxidant activity. Therefore, the methanol leaf extract of *X. caffra* possesses antioxidant activity with the potential to attenuate oxidative stress and confer protection against oxidative stress-mediated diseases and drug toxicities. However, studies of longer duration are needed to further explore the renoprotective potential of the plant extracts. Toxicity studies on *X. caffra* extracts are also required to better guide the choice of optimal doses to be used in other *invivo* studies.

What is already known on this topic:

- *X. caffra* extracts contain various phytochemicals such as phenolic compounds.
- Phytochemicals in *X. caffra*, particularly polyphenols have demonstrated antioxidant activity in various *in vitro* assays but none of the studies investigated the activity *in vivo*.

What this study adds:

- Quantification of phenolics in the leaves of a species of *X. caffra* occurring in Zambia.
- Investigation of the antioxidant effects of *X. caffra* extract in a live model of drug-induced oxidative stress.

Acknowledgments

The authors would like to thank members of the Departments of Physiological Sciences, Chemistry, and Biological Sciences at the University of Zambia, and the Pathology and Chemistry Laboratories at the University Teaching Hospitals, Lusaka, Zambia. Gratitude goes to the family and sponsors of the Moses Sinkala Memorial Scholarly Award for funding the research.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Mutinta Chipuwa: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original draft. **Kaampwe Muzandu:** Validation, Supervision, Writing – reviewing and editing. **Lavina Prashar:** Validation, Supervision, and Writing – review and editing. **Sergio Muwowo:** Methodology, Investigation, Writing – review and editing.

REFERENCES

1. Valko M, Jomova K, Rhodes CJ, Kuča K, Musílek K. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol*. 2016;90(1):1-37.
2. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press; 2015.
3. Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: still a therapeutic

target? *Nutrients*. 2019;11(9):2090.
4. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. *J Toxicol*. 2012;2012:645460.
5. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15(10):7313-52.
6. Akomolafe SF, Akinyemi AJ, Anadozie SO. Phenolic acids (gallic and tannic acids) modulate antioxidant status and cisplatin-induced nephrotoxicity in rats. *ISRN Toxicol*. 2014;2014:973751.
7. Halliwell B. Drug antioxidant effects. *Drugs*. 1991;42(4):569-605.
8. dos Santos NA, Rodrigues MA, Martins NM, Dos Santos AC. Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. *Arch Toxicol*. 2012;86(8):1233-50.
9. Kumar S, Kumar R, Dwivedi A, Pandey AK. In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngonium podophyllum and Eichhornia crassipes leaf extracts on isoniazid-induced oxidative stress and hepatic markers. *Biomed Res Int*. 2014;2014:459019.
10. Perše M, Večerić-Haler Ž. Cisplatin-induced rodent model of kidney injury: characteristics and challenges. *Biomed Res Int*. 2018;2018:1462802.
11. Wojcik M, Burzynska-Pedziwiatr I, Wozniak L. A review of natural and synthetic antioxidants important for health and longevity. *Curr Med Chem*. 2010;17(28):3262-88.
12. Nickrent DL, Malécot V, Vidal-Russell R, Der JP. A revised classification of Santalales. *Taxon*. 2010;59(2):538-58.
13. Chinsembu KC, Syakalima M, Semenya S. Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Lusaka, Zambia. *S Afr J Bot*. 2019;122:369-84.
14. Nyirenda J, Chipuwa M. An ethnobotanical study of herbs and medicinal plants used in Western, Copperbelt, Central and Northern provinces of Zambia. *Phytomedicine Plus*. 2024;4(1):100514.
15. Phiri PS. A checklist of Zambian vascular plants. Lusaka: Southern African Botanical Diversity Network; 2005.
16. Palgrave KC. Palgrave's trees of southern Africa. 3rd ed. Cape Town: Penguin Random House South Africa; 2015.
17. Maroyi A. Ximenia caffra Sond. (Ximeniaceae) in sub-Saharan Africa: a synthesis and review of its medicinal potential. *J Ethnopharmacol*. 2016;184:81-100.
18. Council NR. Guide for the care and use of laboratory animals. 8th ed. Washington, D.C.: National Academies Press; 2010.
19. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Schaumburg: American Veterinary Medical Association; 2020.
20. Agyigra IA, Ejiofor JI, Magaji MG. Acute and subchronic toxicity evaluation of methanol stem-bark extract of Ximenia americana Linn (Olacaceae) in Wistar rats. *Bull Fac Pharm Cairo Univ*. 2017;55(2):263-7.
21. Ajith T, Usha S, Nivitha V. Ascorbic acid and α-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice: a comparative study. *Clin Chim Acta*. 2007;375(1-2):82-6.
22. Ho WY, Liang W, Yeap SK, Beh BK, Yousr AH, Alitheen NB. In vitro and in vivo antioxidant activity of Vernonia amygdalina water extract. *Afr J Biotechnol*. 2012;11(17):4090-4.
23. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16(3):144-58.
24. Masuku NP, Unuofin JO, Lebelo SL. Phytochemical content, antioxidant activities and androgenic properties of four South African medicinal plants. *J Herbmed Pharmacol*. 2020;9(3):245-56.
25. Zhen J, Guo Y, Villani T, Carr S, Brendler T, Mumbengegwi DR, et al. Phytochemical analysis and anti-inflammatory activity of the

extracts of the African medicinal plant Ximenia caffra. *J Anal Methods Chem*. 2015;2015:948150.
26. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239(1):70-6.
27. Aremu OO, Oyedeji AO, Oyedeji OO, Nkeh-Chungag BN, Rusike CRS. In vitro and in vivo antioxidant properties of Taraxacum officinale in Nω-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats. *Antioxidants* (Basel). 2019;8(8):309.
28. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med*. 1990;9(6):515-40.
29. Kappus H. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In: Sies H, editor. Oxidative stress. London: Academic Press; 1985. p. 273-310.
30. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and related aldehydes. *Free Radic Biol Med*. 1991;11(1):81-128.
31. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 1968;125(1):189-98.
32. Makkar HP, Siddhuraju P, Becker K. Plant secondary metabolites. Totowa: Humana Press; 2007.
33. Liu W, Yin D, Li N, Hou X, Wang D, Li D, et al. Influence of environmental factors on the active substance production and antioxidant activity in Potentilla fruticosa L. and its quality assessment. *Sci Rep*. 2016;6:28591.
34. Verma PK, Raina R, Sultana M, Singh M, Kumar P. Total antioxidant and oxidant status of plasma and renal tissue of cisplatin-induced nephrotoxic rats: protection by floral extracts of Calendula officinalis Linn. *Ren Fail*. 2016;38(1):142-50.
35. Akca G, Eren H, Tumkaya L, Mercantepe T, Horsanali MO, Deveci E, et al. The protective effect of astaxanthin against cisplatin-induced nephrotoxicity in rats. *Biomed Pharmacother.* 2018;100:575-82.
36. Ndhlala AR, Mupure C, Chitindingu K, Benhura MA, Muchuweti M. Antioxidant potentials and degrees of polymerization of six wild fruits. *Sci Res Essays*. 2006;1(3):87-92.
37. Oosthuizen D, Goosen NJ, Stander MA, Ibrahim AD, Pedavoah MM, Usman GO, et al. Solvent extraction of polyphenolics from the indigenous African fruit Ximenia caffra and characterization by LC-HRMS. *Antioxidants* (Basel). 2018;7(8):103.
38. Rahim NA, Zakaria N, Dzulkarnain SM, Azahar NM. Antioxidant activity of Alstonia angustifolia ethanolic leaf extract. *AIP Conf Proc*. 2017;1847:020048.
39. Ugur S, Ulu R, Dogukan A, Gurel A, Yigit IP, Gozel N, et al. The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. *Ren Fail*. 2015;37(2):332-6.
40. Hosseinian S, Hadjzadeh MA, Roshan NM, Khazaei M, Shahraki S, Mohebbati R, et al. Renoprotective effect of Nigella sativa against cisplatin-induced nephrotoxicity and oxidative stress in rat. *Saudi J Kidney Dis Transpl*. 2018;29(1):19-29.
41. Packer L, Cadenas E. Hydrogen peroxide and cell signaling, Part B. 1st ed. London: Academic Press; 2013.
42. Ndhlala AR, Muchuweti M, Mupure C, Chitindingu K, Murenje T, Kasiyamhuru A, et al. Phenolic content and profiles of selected wild fruits of Zimbabwe: Ximenia caffra, Artabotrys brachypetalus and Syzygium cordatum. *Int J Food Sci Technol*. 2008;43(8):1333-7.