Original Research Article

EFFECT OF AQUEOUS EXTRACT OF THE AERIAL PARTS OF *LEONURUS CARDIACA* ON CISPLATIN-INDUCED HEPATO-RENAL DAMAGE IN WISTAR ALBINO RATS

Abstract

This study investigated the effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on cisplatin-induced hepato-renal damage in male Wistar albino rats. Sixty male wistar albino rats weighing between 180 and 220g were used for this study. The rats were grouped into 12 groups, five rats per group. Group 1 served as normal control, group 2 received 5mg/kg b.w of cisplatin, serving as negative control while group 3 received 5mg/kg b.w of cisplatin and treated with 25mg/kg b.wt of hydrochlorothiazide and served as positive control. Group 4-12 rats were cisplatin-induced cardiovascular damage treated with *Leonurus cardiaca* extract at 166, 250, and 500mg/kg for 7, 14, and 21 days. All reagents used were of analytical grades. Biochemical assays were determined using standard methods and procedures. The Na^+, Cl^-, HCO_3^-, creatinine, and urea levels of kidney homogenate of group 7 treated rats with the extract at 250mg/kg b.w for 7 days were 123.05±0.01mmol/l, 1.07±0.01mmol/l, 27.27±0.01mmol/l, 13.63±0.01mmol/l, 0.68±0.02mmol/l, and 1.14±0.01mmol/l respectively and were significantly increased when compared to the negative and normal control groups. Similar increases occurred for 14 and 21 days treatments. The kidney homogenate Na^+, Cl^-, HCO_3, creatinine, and urea of group 7 treated rats with the extract at 500mg/kg b.w for 7 days were 158.05±0.01mmol/l, 3.03±0.01mmol/l, 31.13±0.01mmol/l, 19.46±0.01mmol/l, 2.06±0.00mmol/l, and 2.03±0.01mmol/l respectively. Values were significantly increased ($p<0.05$) in comparison to the normal, negative control groups. Similar increases were observed for 14 and 21 days treatments. The plasma ALT, ALP, and AST activities were 187.35±0.00U/L, 98.03±0.01U/L, 185.64±40.81U/L respectively, were significantly decreased ($p<0.05$) in comparison to negative control treated with the extract at 250mg/kg b.w for 14 days. The plasma ALT, ALP, and AST activities of group 10 rats treated with the extract at 500mg/kg b.w for 7 were 164.24±0.01U/L, 87.02±0.01U/L, and 183.74±0.01U/L respectively and were significantly decreased ($p<0.05$) in comparison to negative control. The hepato-renal curative potential of *Leonurus cardiaca* could be attributed in part to its ability in enhancing liver and kidney regeneration

Keywords: *Leonurus cardiaca*; *hepato-renal damage; hepatic and renal biomarkers; cisplatin*

Comment [G1]: Leonurus cardiaca

Comment [G2]: The rats were grouped into 12 groups of five rats per group.

Comment [G3]: Add AQUEOUS EXTRACT

1.0 INTRODUCTION

Kidney damage (KD) is defined as damage or failure of glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for ≥3 months and has become a public health problem, with adverse outcomes of cardiovascular disease and premature death [1]. Hypertension and diabetes mellitus [2,3,4], toxicity of drugs and diagnostic medicaments which are intrinsic to pharmacological compounds, and reactive oxygen species generated through the mechanism of action of anticancer agents and the direct effects of drug metabolites are some of the causes of kidney damage [5,6].

One vital cause of hepatoxicity is unfavorable drug reactions which might require clinical attention or possibly, liver transplant [7]. The liver is a prime target for drug-induced toxicity because it is responsible for concentrating and metabolizing various medicaments. Therapies such as anticancer, antibiotics, antituberculosis agent, antiretroviral, paracemol, and cardiac arrest drugs produce liver toxicity as one of their side effects. [8]. Drug-induced toxicity is classified as acute or chronic, and as hepatitis, cholestatic or a mixed form of disorder [9]. Toxic metabolite (generated by liver cytochrome P450 system through a series of phase I and II reactions) can give rise hepatocyte damage and death by inducing apoptosis or necrosis [10].

Leonurus cardiaca (Motherwort) is probably best known as a uterine stimulant, which is where its name comes from. It is used for painful or delayed periods, and in the last few weeks of pregnancy to prepare for childbirth. It is also known to ease symptoms of menopause [11]. It is

used by trado-medical practioners for the treatment of heart failure in traditional medicine. *Leonurus cardiaca* is a Latin name which means something like "lion's heart," and refers to motherwort's use as a cardiovascular tonic in traditional system of medicine. This study evaluated the effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on cisplatin-induced hepato-renal damage in Wistar albino rats.

2.0 MATERIALS AND METHODS

2.1 Chemical/Reagents

All chemical/reagents used for this study were purchased from commercial industries and the manufacturers standard methods and procedure were strictly followed with regard to this study

2.2 Source and Identification of Plant Material

The fresh aerial parts of *Leonurus cardiaca* (LNC) were harvested from Idema Community, in Ogbia Local Government Area of Bayelsa State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/P/203.

2.3 Aqueous Extraction of the Aerial Parts of *Leonurus cardiaca*

The fresh aerial parts of *Leonurus cardiaca* were harvested from Idema Community Ogbia Local Government Area, Bayelsa State, Nigeria. The aerial parts were washed with clean running tap water and air-dried under shade for five weeks. The dried aerial parts of the plant were pulverized into coarse powder. Three hundred and fifty grams (350 g) of the powdered sample was macerated in 500 ml of distilled water at room temperature for 72 hours. The mixture was

filtered using a Whatzman filter paper grade 1 (542 mm) and the filtrate condensed and evaporated to dryness using a rotary evaporator and water bath at 50˚C. The extract which weighed 85 g was stored in air-tight containers in a refrigerator until when required for treatments.

2.4 Source of Experimental Wistar Albino Rats

Sixty (60) adult male albino Wistar rats (Rattus norvegicus) weighing 180 and 200 g were purchased from the Biochemistry Animal House, University of Nigeria Nsukka (UNN) and be acclimatized for two weeks, giving free access to rat feed and distilled water (DW). The rats were kept in clean plastic cages in well ventilated room, fed with standard animal feeds, produced by Grand Cereals and Oil Mills Ltd., Port Harcourt, and water *ad libitum*. The animals were handled with care, according to the principles and standard protocols for the use of laboratory animals for experiments.

Comment [G4]: In italic

Comment [G5]: Weighing between 180 to 200 g

2.5 Sources of Drug

Cisplatin injection (IP 50 mg/50 ml) was purchased from Everyday Pharmacy LTD Choba, Rivers State (Manufactured by Celon Laboratories PVT. LTD., Plt No:2, ALEAP Industrial Estate Gajularamaram, Medchal District-500090, Telangana, India. Mfg. Lic No: 14/RR/AP/2008/F/CC, Batch NO: CS11977BC. Marketing company: Purdue Pharma L.P., Stamford, CT O6901-3431. Manufacturing date:09/2019, Exp Date:08/2021. Hydrochlorothiazide (25 mg/kg b.wt) was purchased Everyday Pharmacy LTD Choba, Rivers State from Riverland Chemists LTD Hospital Road, Port Harcourt (Manufactured by Janssen-Cilag 2020, marketing company:McNeil consumer Healthcare, Batch N0: 13303, Manufacturing date: 3/02/1017-4/22/2021).

2.6 Experimental Design

Sixty (60) Wistar albino rats weighing between 180 and 200g were used for this study. They were be purchased from the Biochemistry Animal House University of Port Harcourt. The rats were then grouped based on body weight into twelve groups five rats per group and treated as follows:

Group 1. Normal control: Feed + H_2O

Group 2. Negative control: Received 5mg/kg CP (i.p)+ feed for 21 days

Group 3. Positive control: Received 5mg/kg CP (i.p)+ 25mg/kg b.w Hydrochlorothiazide for 21 days

Group 4: 5mg/kg b.w CP (i.p)+166 mg/kg/b.wt Extract+ feed for 7days

Group 5: 5mg/kg b.w CP (i.p)+166 mg/kg/b.w Extract+ feed for 14 days

Group 6: 5mg/kg b.w CP (i.p)+166 mg/kg/b.w Extract+ feed for 21 days

Group 7: 5mg/kg b.w CP (i.p)+250 mg/kg/b.w Extract+ feed for 7days

Group 8: 5mg/kg b.w CP (i.p)+250 mg/kg/b.w Extract+ feed for 14 days

Group 9: 5mg/kg b.w CP (i.p)+ 250 mg/kg/b.w Extract+ feed for 21 days

Group 10: 5mg/kg b.w CP (i.p) +500 mg/kg/b.w Extract + feed for 7 days

Group 11: 5mg/kg b.w CP (i.p) +500 mg/kg/b.w Extract + feed for 14 days

Group 12: 5mg/kg b.w CP (i.p)+ 500 mg/kg/b.w Extract + feed for 21 days

Exactly, 24 hours after the last day of oral treatment with the extract, the rats were humanly sacrificed through cervical dislocation, blood sample were be collected for biochemical assays. The liver and kidney were harvested and were cut into two equal halves for each organ. Half of the kidney were homogenized for estimation of oxidative biomarkers for and kidney, while the other halves were subjected to histological examination.

2.7 Induction of Hepato-Renal Damage

Cisplatin (5mg/kg b.w) was the drug of choice for induction of hepato-renal damage in the rats. It is an injectable liquid manufactured by Kwality Pharmaceuticals Pvt. Ltd., Everyday Pharmaceuticals East-West Road, Port Harcourt, Nigeria.

2.8 Homogenate Preparation

Kidney issues sample (100 mg) was homogenized in 10 volume of 100 mM KH_2PO_4 buffer containing 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 12000×g for 30 min at 4 °C to obtain the supernatant which was then stored at − 20 °C for enzymes assays.

2.9 Biochemical Analysis

Different biochemical parameters were measured using ELISA and Randox kits in a biochemical analyzer. The liver function parameters determined were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate, bilirubin, albumin, and total protein. Kidney homogenate biomarkers determined were sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-), bicarbonate ion (HCO_3^-), creatinine and blood urea nitrogen (BUN). The oxidative

stress biomarkers of homogenate determined were malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT).

2.10 Determination of Hematological Parameters

The microscopic method of slide viewing was employed to determine the WBC count.

Hemoglobin concentration was determined colorimetrically and the concentration of hemoglobin was reported in g/dl. The haematocrit level was determined using the method as described by [12]. The mean corpuscular haemoglobin (MCH) also calculated on hematology analyzers and reported in picograms (pg), is a measure of the average amount of hemoglobin in the RBCs. It was calculated by dividing the hemoglobin (in g/L) by the RBC count. The mean corpuscular volume level was calculated using the formula as described by [13].

2.11 Histological Analyses of Liver and Kidney Tissues

Histopathological examination was carried out based on standard laboratory method. The kidney and liver tissues was fixed in 10% formalin, dehydrated embedded paraffin, sectioned and stained with hematoxlin and eosin. The glacial glass slides were viewed under the microscope at x 40 magnification

2.12 Statistical Analysis

The data were expressed as means ± standard error of the mean (SEM). The analysis was carried out using the Statistical Package for Social Science (SPSS version 20.0). One-way analysis of variance (ANOVA) was done followed by Tukey's post hoc test was applied to compare means among groups at $p \leq 0.05$ level of significance.

3.0 RESULTS AND DISCUSSION

Intraperitoneal administration of 5mg/kg of cisplatin to rats in group 2-12 resulted in a significant decreases on the kidney homogenate sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), creatine and urea concentrations when compared to the positive and negative control values as stated in Table 1. These effects were similar to the report of Martina and Željka [14] on cisplatin-induced rodent model of kidney injury: characteristics and Challenges." Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex (15). Significant differences were not observed after treatment with the extract at 166mg/kg b.wt dose for 7, 14, and 21 days in comparison to the negative control (Table 1), which is reflective that the extract at 166 mg/k did not elicit ameliorative effect against cisplatin-induced kidney damage in the rats. However, significant increases on kidney homogenate sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), creatine and urea concentrations were observed at 250mg/kg b.wt treatment for 7, 14, and 21 days in comparison to the positive and negative control values (Table 1). More so, a more significant increases on the kidney homogenate sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), creatine and urea concentrations were observed at 500mg/kg b.wt treatment for 7, 14, and 21 days in comparison to the positive and negative control values (Table 1). The significant improvement or increases observed following treatment with aqueous extract of the aerial parts of *L. cardiaca* is suggestive of the ameliorative effect of the plant against hepato-renal damage in Wistar rats. This results is in agreement with the report of Chinedu *et al*. [16] on the effects of ethanolic extracts

of leaf, seed and fruit of *Datura metel* and Rana *et al.* (2016) on amelioration of cisplatin-induced nephrotoxicity by ethanolic extract of *Bauhinia purpure.*

Table 1 Effect of aqueous extract of the aerial parts *Leonurus cardiaca* on the biomarkers of kidney homogenate in cisplatin-induced hepato-renal damage in Wistar albino rats

Treatment	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3 (mmol/I)	Creatinine (mmol/l)	Urea (mmol/l)
N/Control	177.34±0.01^{a}	4.24±0.01^{a}	86.55±0.01^{a}	25.75±0.01^{a}	0.50±0.06^{a}	2.63±0.01^{a}
Ne/Control	121.55±0.01^{ab}	2.63±1.72^{ab}	25.74±0.11^{ab}	9.05±0.01^{ab}	0.10±0.00^{ab}	0.74±0.01^{ab}
Po/Control	184.35±0.01^{c}	4.76±0.01^{c}	41.11±0.03^{c}	23.64±0.01^{c}	2.84±0.01^{c}	2.94±0.01^{c}
166mg/kg+CP 7	121.19±0.01^{k}	0.98±0.00^{c}	25.94±0.01^{k}	9.16±0.01^{k}	0.60±0.00^{c}	0.84±0.01^{k}
166mg/kg+CP 14	121.35±0.07^{k}	1.02±0.01^{c}	26.02±0.00^{c}	9.26±0.01^{k}	0.20±0.00^{c}	0.96±0.01^{c}
166mg/kg+CP 21	121.95±0.01^{k}	1.04±0.01^{c}	26.22±0.01^{c}	9.83±0.01^{k}	0.56±0.02^{c}	1.02±0.00^{c}
250mg/kg+CP 7	123.05±0.01^{c}	1.07±0.01^{c}	27.27±0.01^{c}	13.63±0.01^{c}	0.68±0.02^{c}	1.14±0.01^{c}
250mg/kg+CP 14	123.24±0.01^{c}	1.12±0.01^{c}	27.53±0.01^{c}	13.93±0.01^{c}	0.88±0.02^{c}	1.27±0.02^{c}
250mg/kg+CP 21	123.84±0.02^{c}	1.16±0.02^{c}	27.85±0.01^{c}	14.25±0.01^{c}	0.56±0.02^{c}	1.42±0.00^{c}
500mg/kg+CP 7	158.05±0.01^{c}	3.03±0.01^{c}	31.13±0.01^{c}	19.46±0.01^{c}	2.06±0.00^{c}	2.03±0.01^{c}
500mg/kg+CP 14	158.73±0.01^{c}	3.19±0.01^{c}	31.42±0.01^{c}	21.01±0.01^{c}	2.25±0.01^{c}	2.16±0.01^{c}
500mg/kg+CP 21	159.01±0.01^{c}	3.67±0.02^{c}	31.75±0.01^{c}	22.05±0.01^{c}	2.76±0.01^{c}	2.51±0.07^{c}

P/Control: positive control, Ne/Control: negative control, N/Control: normal control. Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript ("k") were not significantly different ($p \leq 0.05$) from the negative control down the groups. Values bearing superscript ("c") were significantly different ($p \leq 0.05$) from the negative control down the groups. Values bearing superscript ("ab") were significantly different ($p \leq 0.05$) from the normal control down the groups.

Significantly decreased mean plasma total protein (TP), albumin (ALB) and increased bilirubin levels occurred after intraperitoneal administration of 5mg/kg b.w of cisplatin to rats in group 3-12 when compared to the negative control (Table 2). Also, the mean alanine amino transferase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) activities were observed to be significantly increased due to intraperitoneal exposure of cisplatin to group 3-12 rats (Table 2) when compared to the negative control. Aspartate transaminase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) are plasma enzymes of hepatic origin which make it possible to study the physiological state of the liver [17, 18]. Total protein, albumin, and total bilirubin are also biomarkers that can be used to predict the health status of the liver. Significant decreases on serum or plasma total protein and albumin concentration as well as increases in bilirubin levels and AST, ALP, GGT, and ALT activities are pointers to liver compromise and hepatotoxicity (19). Reactive oxygen species generated by chemical agents or organic pollutants are the major mechanisms that damage the membranes of hepatic tissues, leading to leakages of proteins and enzymes into the blood [18]. However, the total protein and albumin were significantly increased while bilirubin level was observed to be significantly decreased at 166mg/kg b.w treatment for 7, 14, and 14 days treatment in comparison to the positive and negative control values (Table 2). The ALT, ALP, and AST activities were significantly decreased at 166mg/kg b.wt of the extract for same periods of treatment. Treatment with the extract at 250 and 500mg/kg b.w for 7, 14, and 21 days resulted in significant increase as well as decrease in the mean plasma total protein, albumin and bilirubin concentrations in comparison to the positive and negative control values (Table 2). The significant differences observed following treatment with the aqueous extract particularly at doses 250 and 500 mg/kg b.w are expression of the capacity of the extract to ameliorate the hepatotoxicity facilitated by 5 mg/ 5 ml

of cisplatin exposure. These results conformed to the report of Emmanuel *et al.* [20] on the effects of *Azadirachta indica* leaf aqueous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats.

Table 2 Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on plasma liver biomarkers activities in cisplatin-induce hepato-renal damage in Wistar albino rats

Treatment	TP (g/L)	ALB (mmol/L)	TBIL (µmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)
P/Control	62.13±0.01^{a}	33.94±0.01^{a}	13.06±0.01^{a}	144.35±0.01^{a}	74.35±0.02^{a}	165.76±0..00^{a}
Ne/Control	21.64±0.01^{ab}	9.25±0.03^{ab}	51.50±0.0^{ab}	201.12±0.01^{ab}	98.34±0.1^{ab}	235.16±0.01^{ab}
Po/Control	53.24±0.01^{d}	24.94±0.01^{d}	27.64±0.01^{d}	95.22±0.02^{d}	54.83±0.01^{d}	120.54±0.01^{d}
166mg/kg+CP7	21.94±0.01^{b}	9.54±0.01^{b}	51.42±0.01^{b}	200.04±0.02^{b}	24.64±0.02^{b}	235.02±0.01^{b}
166mg/kg+CP14	22.97±0.01^{b}	9.84±0.01^{b}	51.24±0.01^{b}	195.85±0.01^{b}	98.25±0.01^{b}	233.44±0.01^{b}
166mg/kg+CP21	25.02±0.01^{b}	10.23±0.01^{b}	51.16±0.0^{b}	187.35±0.00^{b}	98.03±0.01^{b}	185.64±40.81^{b}
250mg/kg+CP7	29.26±0.02^{b}	10.65±0.01^{b}	51.03±0.01^{b}	182.93±0.01^{b}	97.03±0.1^{b}	202.45±0.01^{b}
250mg/kg+CP14	32.11±0.01^{b}	10.86±0.01^{b}	48.86±0.01^{b}	175.47±0.01^{b}	93.27±0.01^{b}	194.63±0.01^{b}
250mg/kg+CP21	32.55±0.01^{b}	12.37±0.06^{b}	48.73±0.01^{b}	172.04±0.01^{b}	87.94±0.01^{b}	191.24±0.0^{b}
500mg/kg+CP7	35.62±0.01^{b}	13.048±0.01^{b}	42.83±0.01^{b}	164.24±0.01^{b}	87.02±0.01^{b}	183.74±0.01^{b}
500mg/kg+CP14	42.04±0.01^{b}	15.25±0.01^{b}	37.77±0.02^{b}	164.24±0.01^{b}	83.84±0.01^{b}	173.63±0.01^{b}
500mg/kg+CP21	42.74±0.01^{b}	15.84±0.01^{b}	37.62±0.01^{b}	147.73±0.01^{b}	78.93±0.01^{b}	162.55±0.01^{b}

P/Control: positive control, Ne/Control: negative control, N/Control: normal control. Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript ("b") were significantly different ($p \leq 0.05$) from the negative control. Values bearing superscript ("d") were significantly different ($p \leq 0.05$) from the normal and negative and control groups.

Antioxidants inhibit the formation of free radicals and prooxidants through a mechanism of antioxidant adaptation which is responsible for the signal formation and transport of the appropriate antioxidant to the site of excessive free radical and prooxidant production [21]. Antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase, and phospholipid hydroperoxide glutathione peroxidase, decompose lipid hydroperoxides to alcohols, and glutathione peroxidase and catalase also reduce hydrogen peroxide to nontoxic substances [22]. Lipid peroxidation generates a wide range of products such as malondialdehyde (MDA) which is a biomarker of oxidative stress [23]. In this study, intraperitoneal administration of 5 mg/5 ml of cisplatin to rats in group 2-12 significantly increased and decreased the mean MDA and GSH concentration respectively and significantly caused decreases in the GPx, CAT and SOD activities in comparison to the normal control values (Table 3). The significant increases in the level of lipid peroxidation marker-MDA, was reflective of reduction in the body antioxidant system and decrease body defense mechanism to scavenging the free radicals. Also, the kidney and liver tissues antioxidant enzymes, SOD and CAT, were decreased along with increased MDA in group 2. The significant increased and decrease the mean MDA and GSH concentration respectively is an indication of lipid peroxidation (Table 3). Treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* at 166 mg/kg b.wt, significantly decreased the mean MDA as well increased the mean GSH concentrations. Also, significant increases occurred on the mean GPx, CAT and SOD activities after treatment at 166mg/kg b.w for 7, 14 and 21 days (Table 3) when compared to the negative control (group 2). Treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* at 250 and 500 mg/kg b.wt, significantly decreased the mean MDA as well as caused increase on the mean GSH concentrations. More so, significant increases on the mean GPx, CAT and SOD activities were

observed after treatment with the extract at 250 and500mg/kg b.w for 7, 14 and 21 days (Table 3) in comparison to the group 2. Meantime, treatment with the extract at dose 500 mg/kg b.wt yielded a more significant decreases and increases on the MDA, GSH, GPx, CAT and SOD activities (Table 3). The significant increase and decrease observed on the mean MDA and GSH respectively as well as the decreases on the mean GSH level, GPx, CAT and SOD activities were reflective of increased lipid peroxidation on the membranes of the kidneys facilitated by cisplatin exposure. However, treatment with the extract at 250 and 5000mg/kg b.wt significantly caused decreased on the mean MDA and increased GSH level, GPx, CAT and SOD activities hen compared to the negative control (Table 3), which is reflective of the ability of the extract to detoxify free radicals. *Leonurus cardiaca* showed antioxidant property by mopping up free radical generated by cisplatin exposure, which is a pro-oxidative agents. Researchers have suggested that *Leonurus cardiaca* is a good source of antioxidants in the biological system and its therapeutic effects have been attributed to its ROS scavenging ability. *Leonurus cardiaca* administered groups prior normalized liver and kidney antioxidant enzymes (SOD and CAT) and stabilize non-enzymatic antioxidant GSH level. Thus, the normalization of SOD, CAT, and GSH in KV-treated groups therefore suggested a protective effect of *Leonurus cardiaca* against ROS overproduction induced by cisplatin in the liver and kidneys of the rats.

Table 3 Effect of aqueous extract of *L. cardiaca* on the oxidative stress biomarkers of kidney homogenate in cisplatin-induced hepato-renal damage Wistar albino rats

Treatment	MDA (mmol/l)	GSH (µg/mg protein)	Glutathione peroxidase (IU/g)	CAT (mg/pro.min)	SOD (mg/g)
N/Control	2.26±0.01^{a}	60.05±0.01^{a}	81.74±0.01^{a}	116.44±0.01^{a}	17.42±0.01^{a}
Ne/Control	6.56±0.01^{b}	11.65±0.01^{b}	12.66±0.01^{b}	87.45±0.02^{b}	1.15±0.01^{b}
Po/Control	2.46±0.02^{bc}	72.05±0.01^{bc}	87.38±0.02^{bc}	105.26±0.01^{bc}	14.26±0.02^{bc}
166mg/kg+CP7D	6.12±0.02^{k}	17.53±0.01^{bc}	20.02±0.01^{bc}	53.14±0.02^{bc}	1.74±0.01^{bc}
166mg/kg+CP14D	6.54±0.02^{k}	23.06±0.01^{bc}	20.82±0.01^{bc}	53.93±0.01^{bc}	6.73±0.01^{bc}
166mg/kg+CP21D	6.54±0.02^{k}	23.06±0.01^{bc}	21.83±0.01^{bc}	54.25±0.01^{bc}	1.44±0.01^{bc}
250 mg/kg+CP7D	6.34±0.01^{k}	28.13±0.02^{bc}	46.03±0.01^{bc}	73.23±0.01^{bc}	1.93±0.01^{bc}
250mg/kg+CP14D	5.08±0.02^{bc}	37.10±0.01^{bc}	46.55±0.01^{bc}	76.04±0.02^{bc}	7.19±0.02^{bc}
250mg/kg+CP21D	4.53±0.01^{bc}	36.75±0.02^{bc}	46.94±0.01^{bc}	75.02±0.01^{bc}	6.95±0.01^{bc}
500 mg/kg+CP7D	3.92±0.01^{bc}	42.16±0.01^{bc}	63.42±0.02^{bc}	87.44±0.01^{bc}	9.13±0.01^{bc}
500mg/kg+CP14D	3.51±0.01^{bc}	53.07±0.01^{bc}	67.43±0.01^{bc}	97.93±0.01^{bc}	10.03±0.01^{bc}
500mg/kg+CP21D	3.13±0.02^{bc}	54.94±0.01^{bc}	67.94±0.01^{bc}	89.14±0.01^{bc}	10.54±0.01^{bc}

P/Control: positive control, Ne/Control: negative control, N/Control: normal control. Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript ("bc") were significantly different ($p \leq 0.05$) from the negative control down the groups. Values bearing superscript ("b") were significantly different ($p \leq 0.05$) from the normal control. Values bearing superscript ("k") were not significantly different ($p \leq 0.05$) from the negative control down the groups.

Hematopoietic system is considered as sensitive to investigate the adverse effects of toxicities induced by antineoplastic agents in animals and humans [24]. Yuan *et al*. [24] reported that administration of CP exhibits significant toxic effects in hematological parameters in rats 6 days after treatment, and the pretreatment with daidzein extract showed a significant protection against hematotoxicity. The toxic effects of CP on hematological parameters were confirmed by significant depletion in count of erythrocytes, Hb, thrombocytes, and PCV [25]. The obtained results implicate an etiological connection between anemia disease and CP. It could be demonstrated by different mechanisms showing bone marrow suppression or elevated osmotic fragility of erythrocytes. Thus, treatment with CP might induce anemic disorders due to suppression of hematopoietic tissues, disturbance in erythropoiesis, and elevated erythrocytes reduction because of an impaired erythrocyte membrane permeability and defective iron metabolism [24]. It was already evidenced that chronic administration of CP leads to decrease in thrombocytes and rise in leukocytes. The depletion of platelets may be due to myelosuppression or platelet aggregation [26]. The elevation of white blood corpuscles (WBC) count might be due to infection during CP treatment or cascade of inflammatory reactions. Moreover, it was demonstrated that CP induces oxidative stress injury in human thrombocytes and lymphocytes leads to apoptosis by affecting their life span [27]. In this study, intraperitoneal administration of 5mg/kg b.wt of cisplatin to rats in group 2-12 resulted in significant decreases in the mean plasma RBC, Hb, haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and WBC (Table 4). The significantly decreased plasma RBC, Hb, haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and WBC observed in group 2 were reflective of cisplatin-induced hematotoxicity. However, treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* at 166, 250, and

500mg/kg b.wt for 7, 14, and 21 days, resulted in significant increases on the mean plasma RBC, Hb, haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and WBC in comparison to the negative control values (Table 4). The significant increases observed on the mean plasma RBC, Hb, haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and WBC after treatment with the extract at 166, 250, and 500mg/kg b.wt for 7, 14, and 21 days are reflective of the ameliorative effect of the extract against cisplatin-induced hematotoxicity in rats. These results agrees with the report of Sanjiv and Jagadish [28] on the effect of daidzein on cisplatin-induced hematotoxicity and hepatotoxicity in experimental rats.

Table 4 Effect of aqueous extract of aerial parts of *L. cardiaca* on the plasma haematological parameters in normal and cisplatin—induced heato-renal damage in Wistar albino rats

Treatment	RBC ($X 10^{12}$/L)	Hb (Mg/dl)	HT (l/L)	MCV (fl)	MCH (pg)	WBC ($X 10^{9}$/L)
N/Control	6.12 ± 0.01^{a}	12.93 ± 0.01^{a}	0.74 ± 0.01^{a}	47.24 ± 0.01^{a}	21.72 ± 0.01^{a}	10.54 ± 0.02^{a}
Ne/Control	1.50 ± 0.03^{k}	4.26 ± 0.01^{k}	0.01 ± 0.00^{k}	78.24 ± 0.01^{k}	53.73 ± 0.01^{k}	2.34 ± 0.01^{k}
Po/Control	15.76 ± 0.02^{b}	11.45 ± 0.01^{b}	0.82 ± 0.01^{b}	41.36 ± 0.01^{b}	18.64 ± 0.01^{b}	17.43 ± 0.0^{b}
166mg/kg+CP 7 D	2.02 ± 0.01^{b}	5.05 ± 0.01^{b}	0.22 ± 0.01^{b}	78.03 ± 0.01^{b}	53.33 ± 0.01^{b}	4.26 ± 0.01^{b}
166mg/kg+CP 14 D	$2..15\pm0.01^{b}$	5.23 ± 0.01^{b}	0.26 ± 0.01^{b}	77.62 ± 0.01^{b}	51.10 ± 0.02^{b}	4.76 ± 0.02^{b}
166mg/kg+CP 21 D	2.44 ± 0.02^{b}	5.35 ± 0.01^{b}	0.35 ± 0.01^{b}	77.25 ± 0.01^{b}	48.24 ± 0.02^{b}	4.89 ± 0.03^{b}
250mg/kg+CP7	6.13 ± 0.01^{b}	6.25 ± 0.01^{b}	0.53 ± 0.01^{b}	58.56 ± 14.64^{b}	31.63 ± 0.01^{b}	7.04 ± 0.03^{b}
250mg/kg+CP 14 D	$2..15\pm0.01^{b}$	6.53 ± 0.01^{b}	0.45 ± 0.01^{b}	58.56 ± 14.6^{b}	31.20 ± 0.02^{b}	7.15 ± 0.02^{b}
250mg/kg+CP 21 D	6.93 ± 0.01^{b}	6.72 ± 0.01^{b}	0.52 ± 0.02^{b}	70.82 ± 0.01^{b}	28.74 ± 0.02^{b}	7.35 ± 0.02^{b}
500mg/kg+CP7	9.93 ± 0.01^{b}	9.04 ± 0.01^{b}	0.62 ± 0.01^{b}	65.17 ± 0.01^{b}	14.36 ± 0.01^{b}	13.82 ± 0.0^{b}
500mg/kg+CP 14 D	11.53 ± 0.01^{b}	9.64 ± 0.02^{b}	0.66 ± 0.01^{b}	62.05 ± 0.01^{b}	14.63 ± 0.01^{b}	13.93 ± 0.0^{b}
500mg/kg+CP 21D	13.21 ± 0.02^{b}	7.96 ± 1.99^{b}	0.72 ± 0.01^{b}	53.73 ± 0.01^{b}	14.93 ± 0.01^{b}	14.22 ± 0.0^{b}

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with superscript (''b'') letters indicate significant differences ($p \leq 0.05$) from the negative control. Values with superscript (''k'') letters indicate significant differences ($p \leq 0.05$) from the normal control.

Both the kidney and liver serve the body as natural filter of blood and remover of drugs or toxic waste products from the body [29]. They produce hormones and maintain the production and metabolism of prostaglandins via cyclooxygenase (COX), especially the kidney. Both organs also participated in the hematopoietic system functions [30]. All these basic functions of the kidney and liver are necessary for homeostasis. Liver and kidney injuries (Nephrotoxicity and hepatotoxicity) arise from their involvement in metabolism, detoxification, storage, and excretion of drugs and their metabolites, making them important target organs for drug-induced injuries [31].

However, histological analysis of the kidneys from negative control rats showed distorted renal corpuscles with hyper-infiltration of the glomerulus and increased mesangial matrix as well as severe and widespread of necrosis of the renal tubules (particularly proximal tubules), which lead to loss of tubular cellular constituents, with dilatation of renal vessels and tubular cell desquamation and intraluminal cast formation and infiltration of inflammatory leukocytes (plate 2) when compared to the positive control histology (plate 1). Histological analysis of the kidneys from LNC-treated rats (166mg/kg LNC+ CP, 250mg LNC+CP, 500mg/kg LNC + CP, and 25mg HT + CP) for 7, 14, and 21 days, showed less histopathological renal alteration (plate 4-12)

Liver sections of the positive control showed normal hepatic cells with usual morphology (plate 13). However, the liver of cisplatin-treated rats had severe periportal congestion, bile duct

proliferation in portal area, portal cellular infiltration, and diffused hydropic degeneration of hepatocytes (plate 14). Histological analysis of the livers tissue of rats in group 4-12 treated with the extract at the stated doses for 7, 14, and 21 days, showed less histopathological hepatic alteration (plate 15-24).

The histopathological evident from both the liver and kidney tissues in this study supported the biochemical results. Thus, *Leonurus cardiaca* to ameliorated the structural and functional integrity of both liver and kidney tissues in cisplatin-induced hepato-renal damage almost restoring them to the normal control (plate 1 and 13), indicating regenerative potential of the extract on damaged liver and kidney tissues.

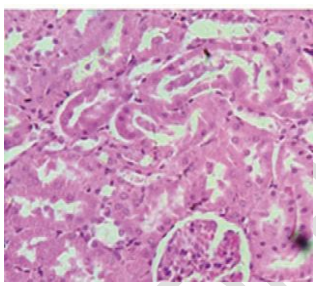

Plate 1: N/Control

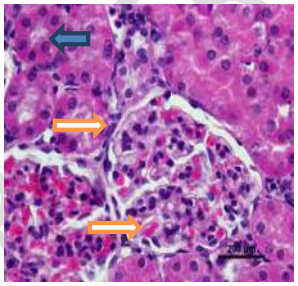

Plate 2: Ne/Control

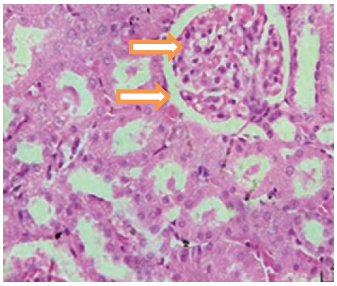

Plate 3: positive control treated 21 days

Plates 1, 2, and 3: Photomicrographs of kidney tissue of normal, negative and positive control. X 400 (H and S) staining

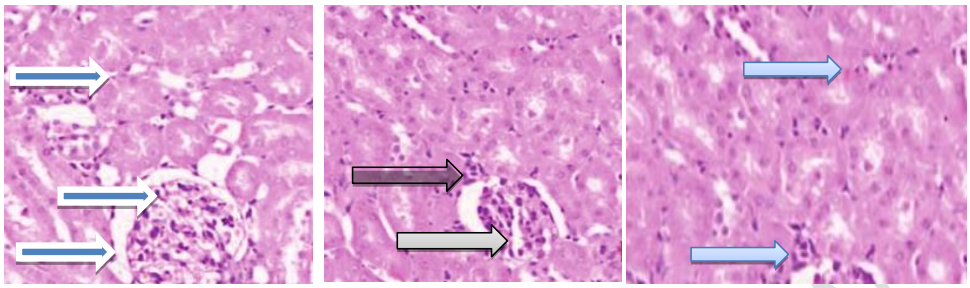

Plate 4: 166mg/kg treated 7 days Plate 5: 166mg/kg treated 14 days Plate 6: 166mg/kg treated 21 days

Plates 4, 5, and 6: Photomicrographs of kidney tissue treated at 166mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining

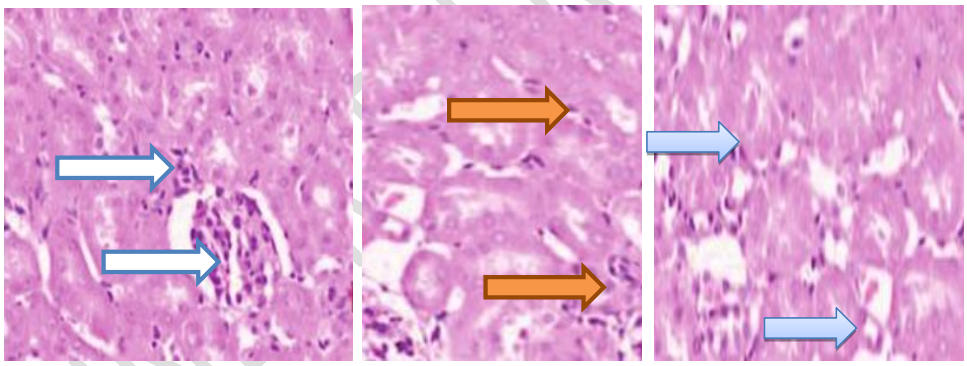

Plate 7:250mg/kg treated 7 days Plate 8: 250mg/kg treated 14 days Plate 9:250mg/kg treated 21 days

Plates 7, 8, and 9: Photomicrographs of kidney tissue treated at 250mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining

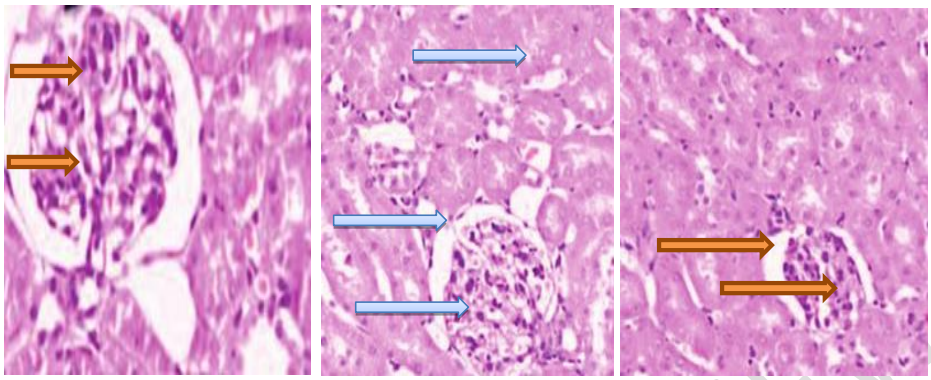

Plate 10:500mg/kg treated 7 days Plate 11:500mg/kg treated 14 days Plate 12: 500mg/kg treated 21 days

Plates 10, 11, and 12: Photomicrographs of kidney tissue treated at 166mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining

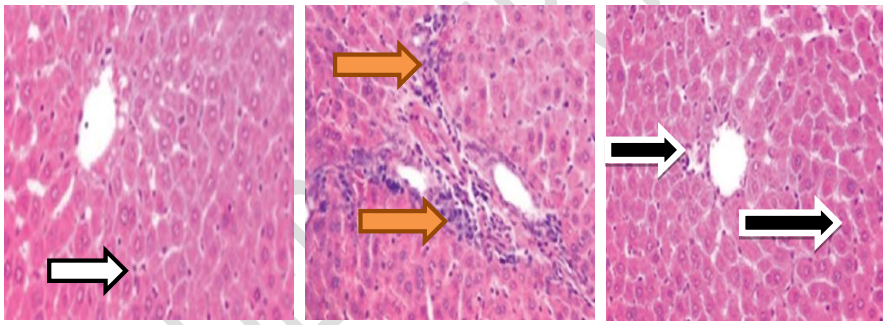

Plate 13:N/C Plate 14:Ne/C Plate 15: Positive control 21 days

Plates 13, 14, and 15: Photomicrographs of liver of the normal, negative and positive control, X 400 (H and S) staining

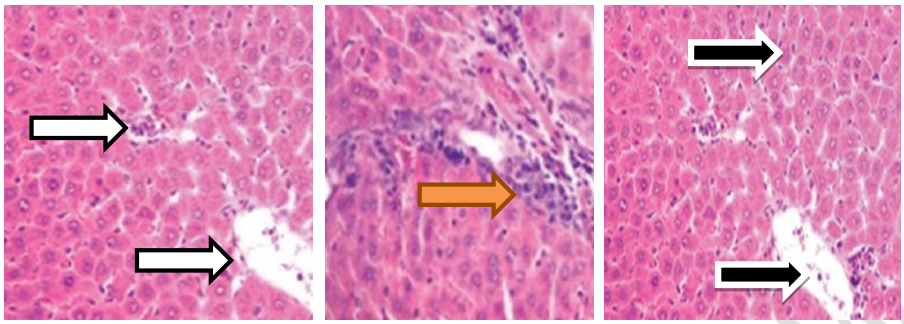

Plate 16:166mg/kg 7 days treated Plate 17:166mg/kg 14 days treated Plate 18: 166 mg/kg 21 days treated.

Plates 16, 17, and 16: Photomicrographs of kidney tissue treated at 166mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining

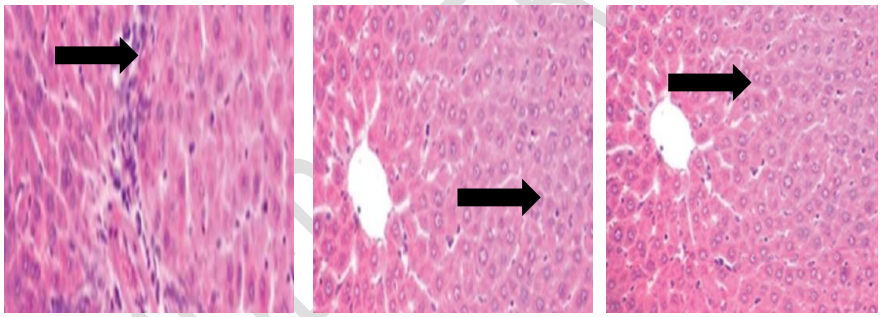

Plate 19:250 mg/kg 7 days treated Plate 20: 250 mg/kg 14 days treated Plate 21: 250mg 21 days treated.

Plates 19, 20, and 21: Photomicrographs of kidney tissue treated at 250mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining.

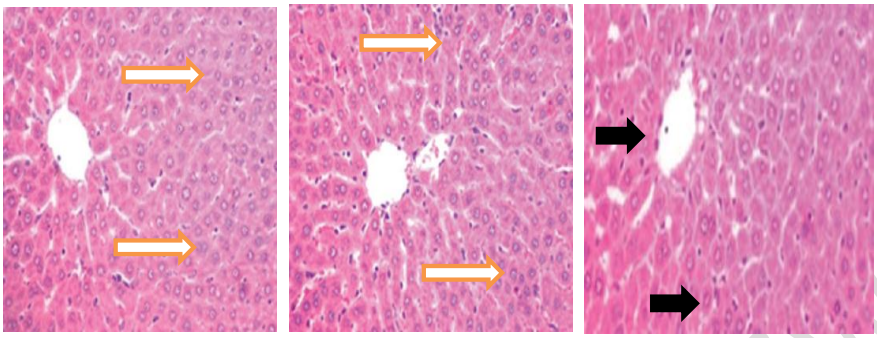

Plate 22:500mg/kg 7 days treated Plate 23:500mg/kg 14 days treated Plate 24:500mg/kg 21 treated.

Plates 22, 23, and 24: Photomicrographs of kidney tissue treated at 500mg/kg b.w for 7, 14, and 21 days , X 400 (H and S) staining.

CONCLUSION

This study revealed that *Leonurus cardiaca* extract ameliorated the damage caused by cisplatin on the kidney and liver tissues through the free radical scavenging potential of the extract.

ETHICAL APPROVAL

All authors hereby declared that the principles of laboratory animal care were followed as well as scientific national laws where applicable. All experiments and procedures were thoroughly examined and approved by the ethical committee on human and animal research University of Port Harcourt.

NOTE

This study highlighted the effectiveness of "traditional medicine" which is an ancient tradition practiced in some parts of India. This ancient concept should be carefully investigated in the light of modern clinical science and can be adopted partially if considered appropriate

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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